

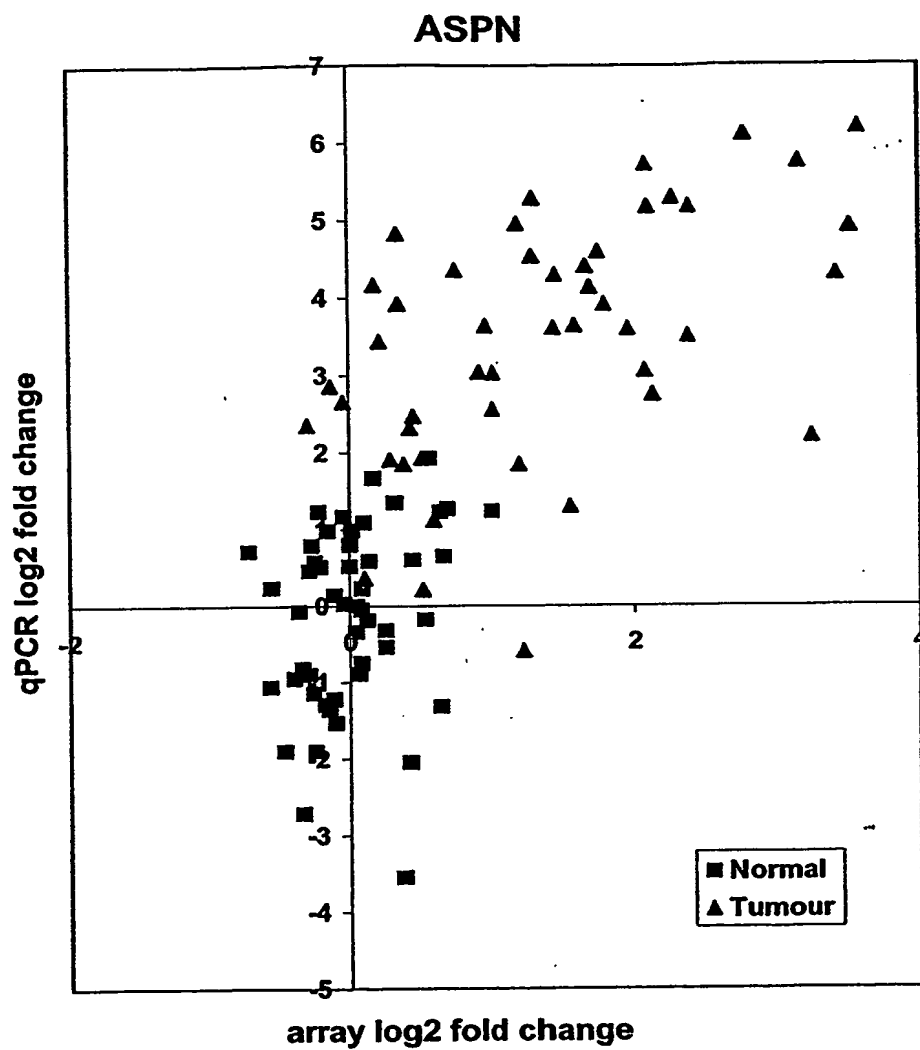
name	symbol	Applied Biosystems "assay on demand" assay #	forward primer	Seq ID No.	reverse primer	Seq ID No.	probe	Seq ID No.
asporin (lrr class 1)	ASPN		AAATCAAAAGGACATTCAAAGGA	1	TGCTTCTGCAATTCGTATATGA	23	TTGGAAATGAGTGCAAAACCTCTTGTATATATG	45
chondroitin sulfate proteoglycan 2 (version)	CSG2		GCCAGTGGATGATGTGTC	2	TCTTGGCATTTTACACAGAGG	24	AGGAAACAGTTGCTTGGGCGCAG	46
cystatins SN, SA & S	CST1, 2, 4		AGTCCGACGCCAAGCTTGA	3	GGGCACTTGTAGATCTGGAAAGA	25	AGCCCAAGTCTGAGAGAAACAGTTGTGC	47
gemma-glutaryl hydrolase	GGL		GTGGCAATGCGGCTTGA	4	TGACAGCAACTCTAGTAGGAAA	26	TTCACTGGAGGTCAATTCACAGCAGAT	48
heparin-like growth factor binding protein 7	HGBP7		CAGGTCAAGAGGCGCACC	5	TCAAGCTCTAGTACAGCTGGG	27	AGCAAGTCTCTTCACTAGTAGAGGCC	49
keratin 10	KLK10		ACAACATGATGTCTGGAAGTGG	6	AGAGAGATGCTTGGAGGGT	28	CTTGCCAGAGTGAAGTCTGGAGGCC	50
leupine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1		CTTGAGTACAAGGCTGAGCTTTC	7	CCGTGACACAGTTCTGCTTACAG	29	CCATCACAGATCATATACATCAAGTCTCTCA	51
lumican	LUM		GATCTGTCCATAGTSCATCTGC	8	CCAATCAATGCCAGGAGAGA	30	TAAGGATTCAAAACCATTTGCCAAAAATGAGTCTAA	52
lyso oxidase-like 2	LOXL2		AGGCCAGCTTCTGCTTGA	9	CCCTGATGCCGAGTTG	31	CGTAAATCTCTGGATGTCTCTCTTCACTCTG	53
matrix metalloproteinase 12	MMP12		GCCTCTGCTGATGACATAGT	10	AGTGACAGCATCAAAAGCTCAATTG	32	TCAGTCCCTGTATGGAGAGCCCAAGAGAA	54
metalloproteinase inhibitor 1	THMP1		CCAGACCACTTATACACGG	11	GGAGCTGTGAGTATCGGC	33	CAAGTGAACAAGATGTATAAGGGTTCACAGC	55
n-acetylglucosaminidase	ASAH1		CGCAGGAGCCCTGCAAA	12	ACAGGACATCATATGTTTCAAA	34	TGCTGTGAACCGCACAGCCAGAGAGATA	56
secreted frizzled-related protein 2	SFRP2		CGCTAGACGCGACCACT	13	TTTTCAGGCTTCACTACCTTT	35	CTGCCAGCCACAGAGAGAGCTC	57
secreted protein, acidic, cysteine rich	SPARC		TCCTCTGTACATCTGGAGTTC	14	GAAAAGCGGCTGTGTC	36	TTGGACACAGACCCCATTTGACGG	58
serpine protease 11 (IGF binding)	PRSS11		TCGGGAGGCCGTTAGTAA	15	AAGGAGATTCAGCTGTCACTTC	37	AGTGTAAATTCGAATCACTTCACCTCCAGG	59
thrombospondin 2	THBS2		TGGAAAGGACTACAGGCCCTATAG	16	TAGTTTGGTCTATAGATGCTCTGAGT	38	AGGCCCAAGACCGGCTACATCAGATC	60
thyroglobulin	TG		GAGCGTCTCTCGCGATGAA	17	TGTAAACCGCTCACTTCAAT	39	TCTGGCAGATTCGATGCCCAAA	61
human cell growth regulator with EF hand domain 1	GRI1		CTGCCCAACCTTCCA	18	TTCTGTCTCTCTAGTCCCTTAGG	40	TCAGGCCAGGAGAGCTCGG	62
human serine or cysteine proteinase inhibitor, clade B, transforming growth factor β1	SERPINE5		TCACGCGATTTTCAGGATAA	19	AAGCGAATTTCTAGTTGCA	41	TGACTCCAGGCCCGCAATGGA	63
human proprotein convertase subtilisin/kexin type 5	TGFβ1		GGTCCATGTCATCAACATGTT	20	TCTGCAAGTTCACTCCCTCTT	42	CAGCTCCAGCCCAACAGACTCAGG	64
matrix metalloproteinase 2	PCSK5		AAAATCTTTGCGGGAATGC	21	TAGTCTGCGCTTGAATACC	43	ACAGAAATGAGGATGAGTAAAGCTGCA	65
human serine or cysteine proteinase inhibitor, clade H, adiccan	MMP2		TTGATGGATCGCTCAGATC	22	TGTCAAGTGGGCTCAGAT	44	TTCAAGGACCGGCTTCAATTGGCG	66
serpin	SERPINH1	Hs00241844 ml						
serpin		Hs00377849 ml						
serpin		Hs00213545 ml						
secreted frizzled-related protein 4	SFRP4	Hs00180066 ml						
inhibin beta A chain	INHBA	Hs00170103 ml						
osteonectin	SPP1	Hs00167093 ml						
transforming growth factor β-induced	TGFβ1	Hs00165908 ml						

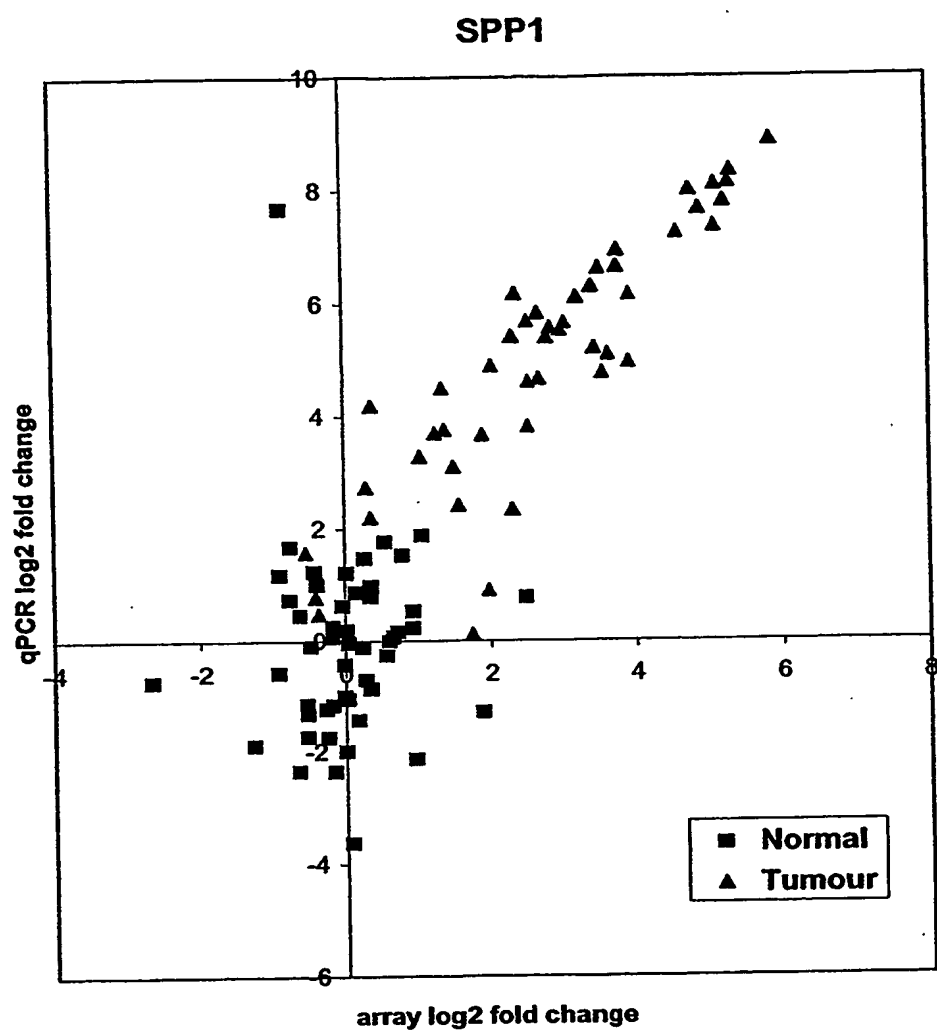
Figure 1

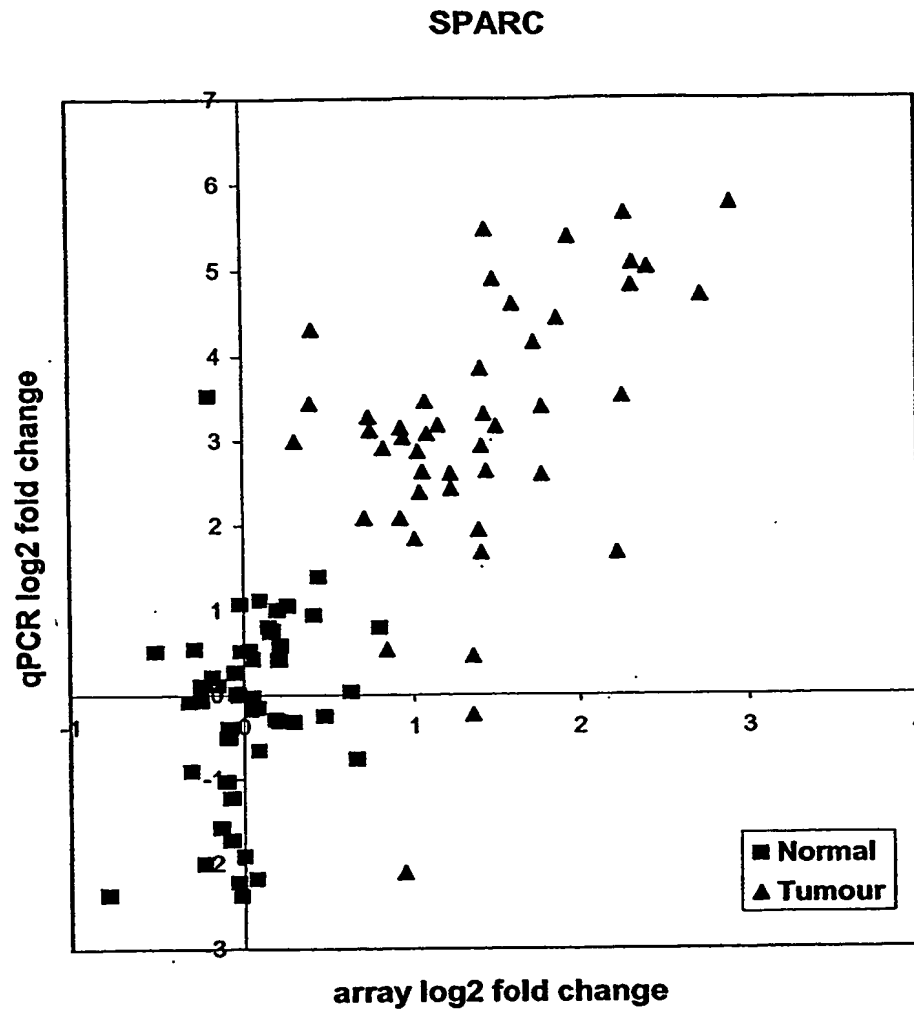
Microarray - Identification of Markers for Gastric Malignancy										fold change	fold change rank	original t-test	Bonferroni-adjusted p value	2 sample Wilcoxon test
name	symbol	MWG oligo #	NCBI mRNA ref sequence	protein ref sequence	fold change	fold change rank	original t-test	Bonferroni-adjusted p value	2 sample Wilcoxon test					
adipon	-	C:0531	NM_015419	NP_056234	1.8	-17818	1.0E-28	3.04E-24	0.0E+00					
asporin (Irr class 1)	ASPIN	A:07749	NM_017680	NP_060150	2.6	-22292	6.4E-23	1.9E-18	0.0E+00					
carboxypeptidase N	CPN2	B:4922	-	P22792	2.7	-22367.5	2.3E-42	7.0E-38	0.0E+00					
cell growth regulatory factor with EF-hand domain	CGR11	A:07876	NM_006569	NP_006560	3.0	-21188.5	4.33E-42	1.3E-37	0.0E+00					
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	A:10008	NM_004385	NP_004376	2.3	-21606.5	2.23E-33	6.65E-29	0.00E+00					
Cystatin SN	CST1	A:06089	NM_001898	NP_001889	2.1	-17475	1.3E-18	3.8E-14	0.0E+00					
Cystatin SA	CST2	A:06089	NM_001322	NP_001313	2.1	-17475	1.3E-18	3.8E-14	0.0E+00					
Cystatin S	CST4	A:06089	NM_001899	NP_001890	2.1	-17475	1.3E-18	3.8E-14	0.0E+00					
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	A:09072	NM_016938	NP_058634	2.4	-22761	2.0E-35	5.9E-31	0.0E+00					
gamma-glutamyl hydrolase	GGH	A:03601	NM_003878	NP_003869	1.6	-18092	1.6E-07	4.8E-03	5.7E-11					
Inhibin beta A chain	INHBA	A:02189	NM_002192	NP_002183	2.1	-21247	1.4E-30	4.3E-26	0.0E+00					
Insulin-like growth factor binding protein 7	IGFBP7	A:03385	NM_001553	NP_001544	3.0	-25854	5.4E-31	1.6E-26	0.0E+00					
kallikrein 10	KLK10	A:07907	NM_002776	NP_002767	2.3	-17986.5	5.0E-10	1.5E-05	4.9E-06					
leudne proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1	A:04646	NM_022356	NP_071751	1.7	-18019	8.2E-14	2.4E-09	1.1E-12					
lumican	LUM	A:09199	NM_002345	NP_002336	2.9	-24927	4.2E-24	1.3E-19	0.0E+00					
lysyl oxidase-like 2	LOXL2	A:06085	NM_002318	NP_002309	1.6	-16994.5	5.9E-10	1.7E-05	7.9E-10					
matrix metalloproteinase 2	MMP2	A:06749	NM_004530	P08253	1.8	-18710	1.2E-11	3.6E-07	1.5E-10					
matrix metalloproteinase 12	MMP12	A:01762	NM_002426	NP_002417	2.1	-20209.5	2.2E-12	6.6E-08	4.9E-11					
metalloproteinase inhibitor 1	TIMP1	A:08048	NM_003254	NP_003245	3.2	-24177	7.5E-38	2.3E-33	0.0E+00					
n-acylsphingosine amidohydrolase	ASAH1	A:10030	NM_004315	NP_004306	1.7	-19636.5	9.6E-16	2.9E-11	0.0E+00					
olfactomedin	OLFM1	B:3555	NM_014279	NP_055094	3.9	-25782.5	6.5E-46	1.9E-41	0.0E+00					
osteopontin	SPP1	A:09441	NM_000582	NP_000573	7.0	-26668	4.0E-32	1.2E-27	0.0E+00					
human proprotein convertase subtilisin/kexin type 5	PCSK5	A:00704	NM_006200	Q92824	1.7	-18736	2.0E-11	6.0E-07	7.3E-11					
group xiii secreted phospholipase a2	PLA2G12b	B:1811	NM_032562	NP_115951	3.0	-23212	7.92E-39	2.36E-34	0.00E+00					
secreted frizzled-related protein 2	SFRP2	B:1634	XM_050625	XP_050625	2.1	-19217	2.7E-10	8.1E-06	4.1E-08					
secreted frizzled-related protein 4	SFRP4	A:07398	NM_003014	NP_003005	3.0	-22153	6.0E-24	1.8E-19	0.0E+00					
secreted frizzled-related protein 4	SFRP4	A:07398	NM_003014	NP_003005	3.0	-22153	6.0E-24	1.8E-19	0.0E+00					
serine (or cysteine) proteinase inhibitor clade H	SERPINH1	A:08615	NM_001235	NP_001226	1.9	-20252	2.8E-34	8.2E-30	0.0E+00					
human serine or cysteine proteinase inhibitor clade B	SERPINB5	A:10485	NM_002639	P36952	1.5	-17026	4.6E-06	1.4E-01	5.6E-06					
serine protease 11 (IGF binding)	PRSS11	B:1274	NM_002775	NP_002766	1.6	-17184.5	9.3E-18	2.8E-13	0.0E+00					
secreted protein, acidic, cysteine rich	SPARC	A:08092	NM_003118	NP_003109	2.5	-22947.5	1.5E-44	4.6E-40	0.0E+00					
spondin 2	SPON2	B:2543	NM_012445	NP_036577	2.4	-20390.5	2.9E-31	8.5E-27	0.0E+00					
stannin	SNN	A:09316	NM_003498	NP_003489	2.1	-20162.5	3.25E-24	9.71E-20	0.00E+00					
thrombospondin 2	THBS2	B:9017	NM_003247	NP_003238	2.6	-22095	5.8E-29	1.7E-24	0.0E+00					
thrombospondin repeat containing 1	TSRC1	B:7686	NM_019032	NP_061905	2.6	-22608	1.38E-45	4.1E-41	0.0E+00					
thyroglobulin	TG	B:5402	NM_003235	NP_003226	2.4	-23644	4.39E-36	1.3E-31	0.0E+00					
transforming growth factor beta-induced	TGFB1	A:08124	NM_000358	NP_000349	2.5	-23339.5	1.96E-24	9.71E-20	0.0E+00					
transforming growth factor beta1	TGFB1	A:07050	NM_000660	P01137	1.6	-17214	2.30E-18	6.86E-14	0.0E+00					
hyaluronan and proteoglycan link protein 4	HAPLN4	C:6300	NM_023002	NP_075378	3.4	-23516.5	7.32E-44	2.2E-39	0.0E+00					
Figure 2														

Quantitative RT-PCR - Quantification of Expression of Selected Gastric Cancer Candidate Genes				
name	symbol	median T:N fold change	Maximum T:N fold change	% T >95th percentile
adipon		5	37	74
asporin (lrr class 1)	ASPN	12	73	91
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	6	24	78
cystatins SN, SA & S	CST1, 2, 4	525	25532	100
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	3	15	56
gamma-glutamyl hydrolase	GGH	5	36	67
Inhibin beta A chain	INHBA	34	357	98
insulin-like growth factor binding protein 7	IGFBP7	4	19	80
kallikrein 10	KLK10	5	633	70
leucine proline-enriched proteoglycan 1(leprecan 1)	LEPRE1	4	17	72
lumican	LUM	5	47	80
lysyl oxidase-like 2	LOXL2	6	26	93
matrix metalloproteinase 12	MMP12	9	586	67
metalloproteinase inhibitor 1	TIMP1	8	19	91
n-acylsphingosine amidohydrolase	ASAH1	3	7	63
osteopontin	SPP1	40	481	96
secreted frizzled-related protein 2	SFRP2	5	85	63
secreted frizzled-related protein 4	SFRP4	56	600	100
secreted protein, acidic, cysteine rich	SPARC	9	56	93
serine protease 11 (IGF binding)	PRSS11	4	25	54
thrombospondin 2	THBS2	25	239	91
thyroglobulin	TG	5	153	54
transforming growth factor B-induced	TGFB1	7	204	82
1 percentage of tumors with expression levels greater than the 95th percentile of non-malignant samples.				

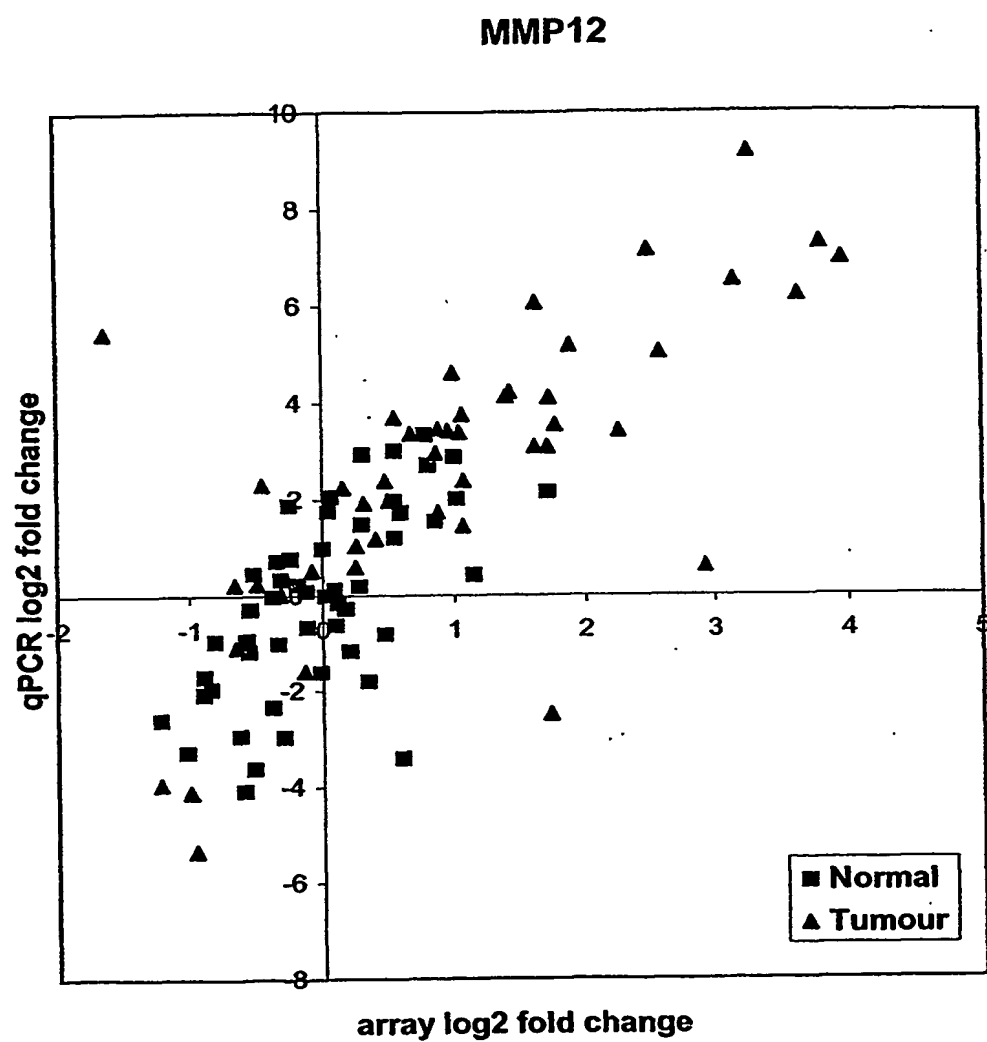
Figure 3

**Figure 4(a)**

**Figure 4(b)**

**Figure 4(c)**

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**Figure 4(d)**

ASPEN-tumor:median normal log2 fold change

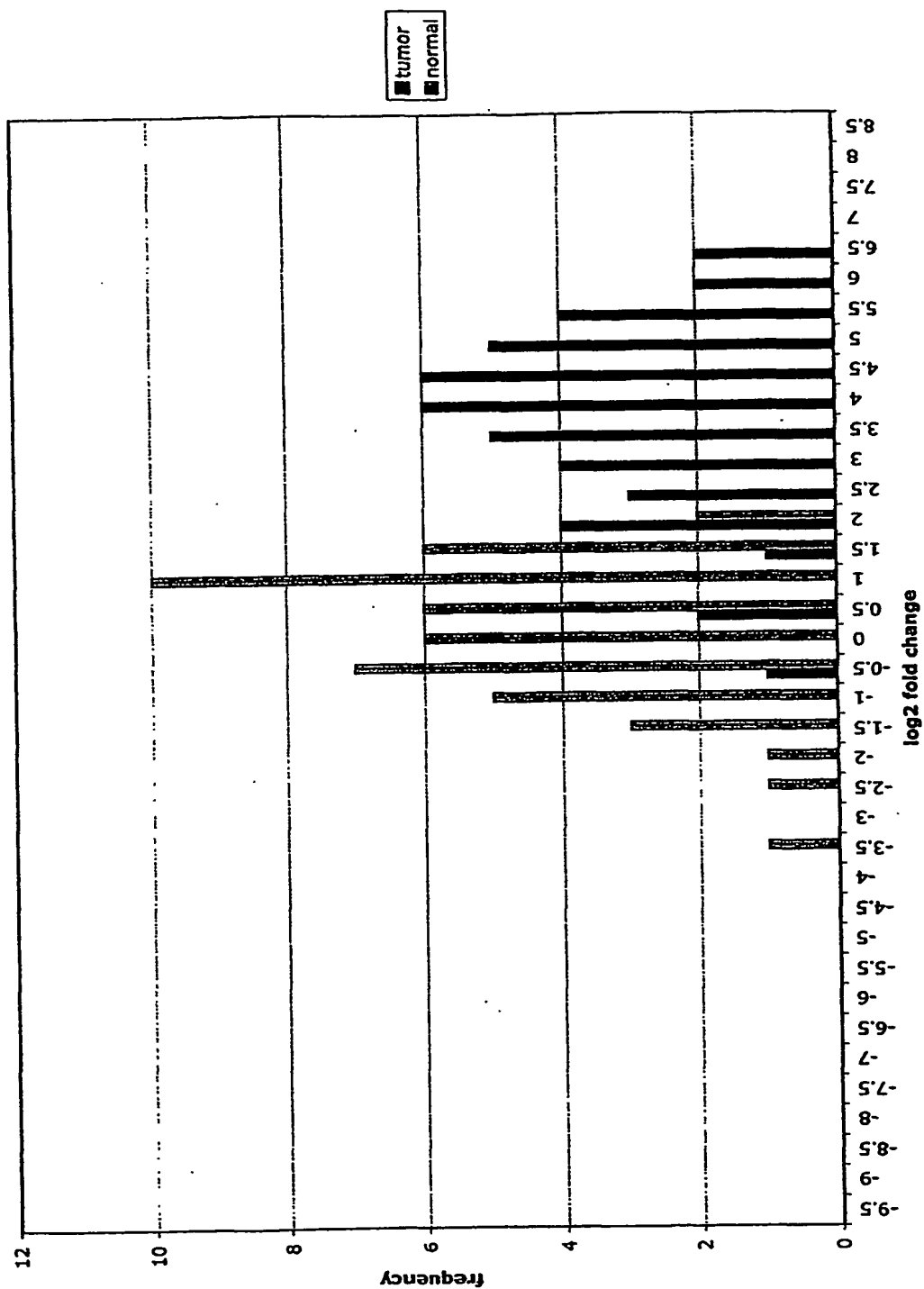


Figure 5(a)



CST1,2 & 4-tumor:median normal log2 fold change

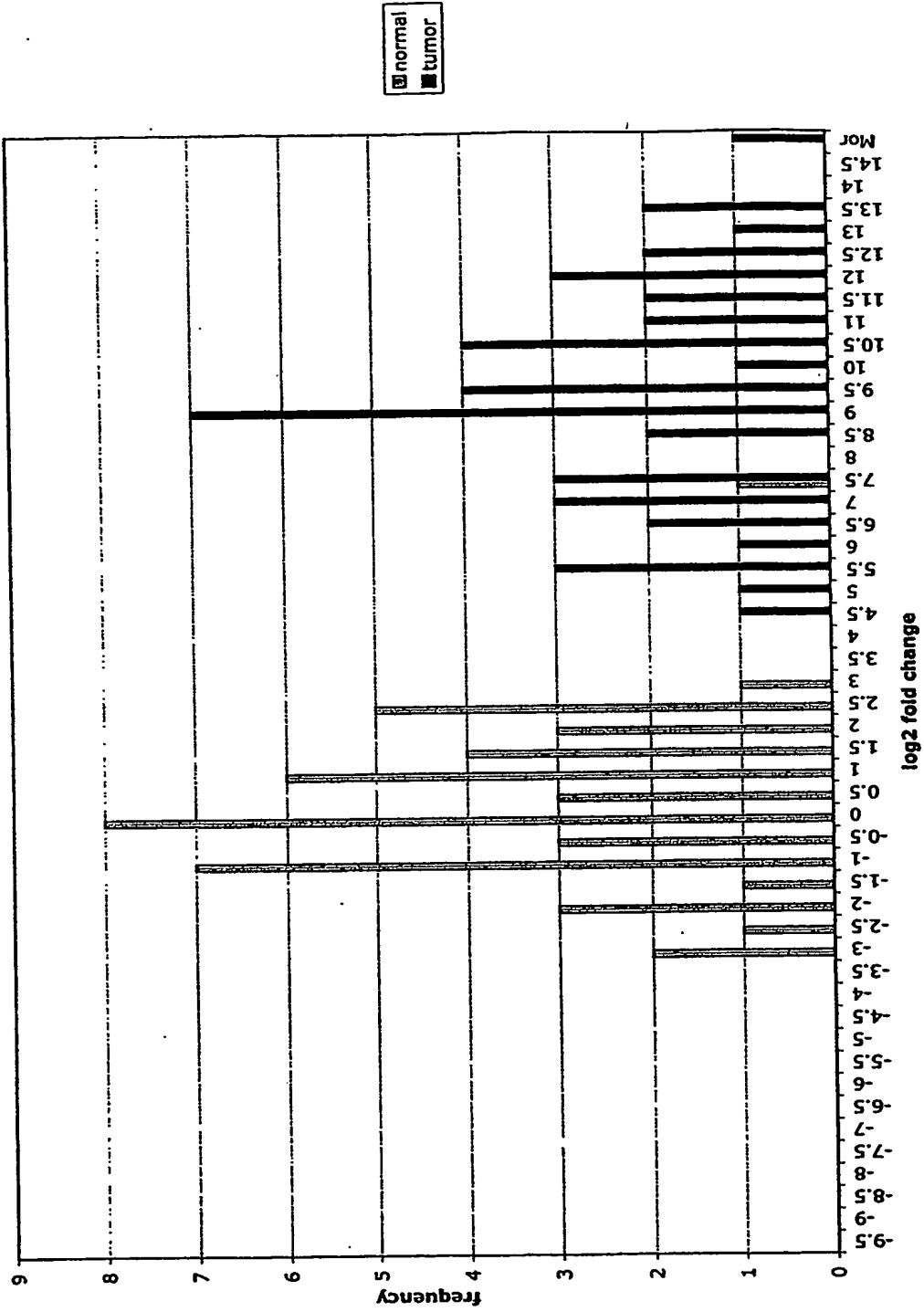


Figure 5(b)

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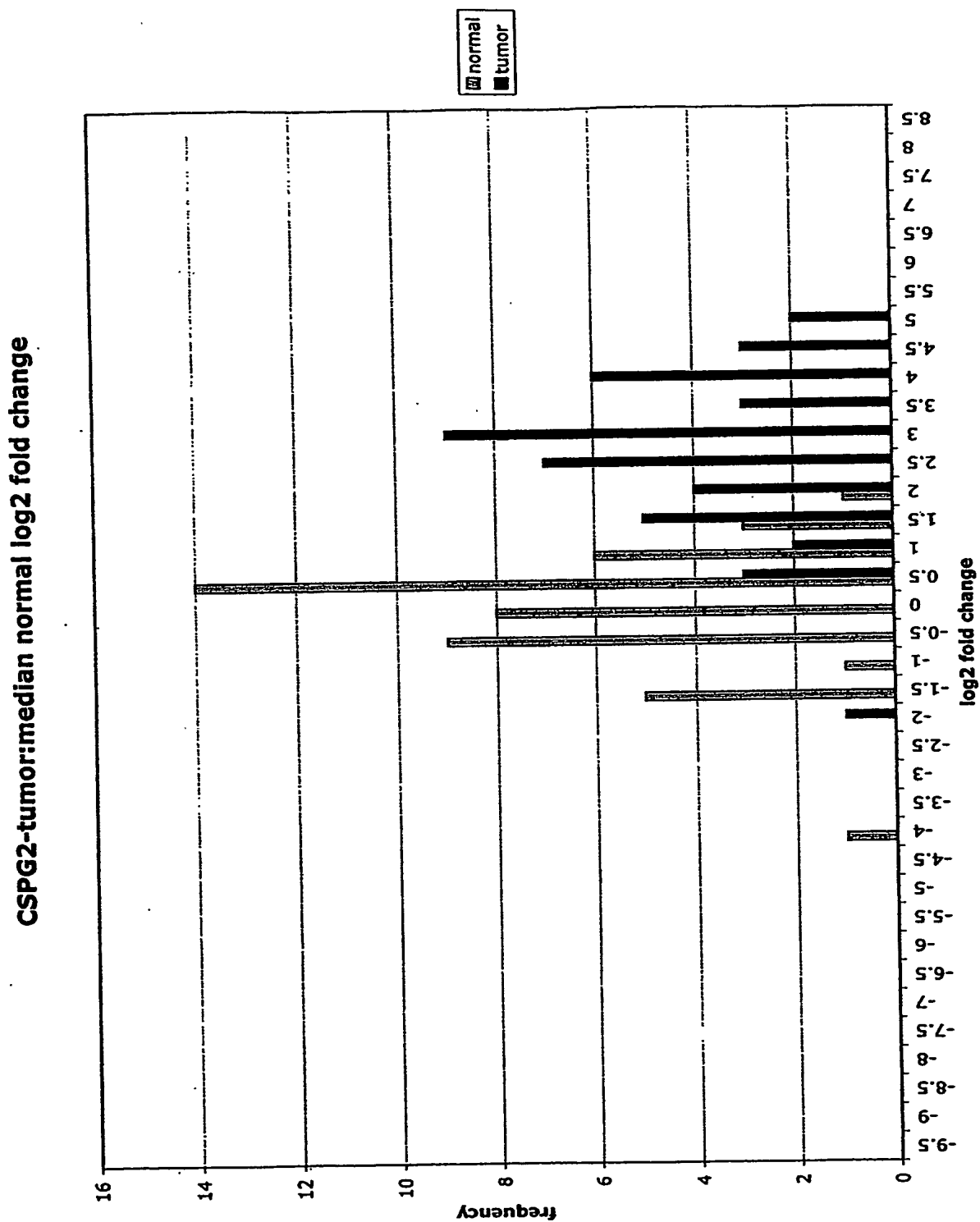


Figure 5(c)

IGFBP7-tumor:median normal log2 fold change

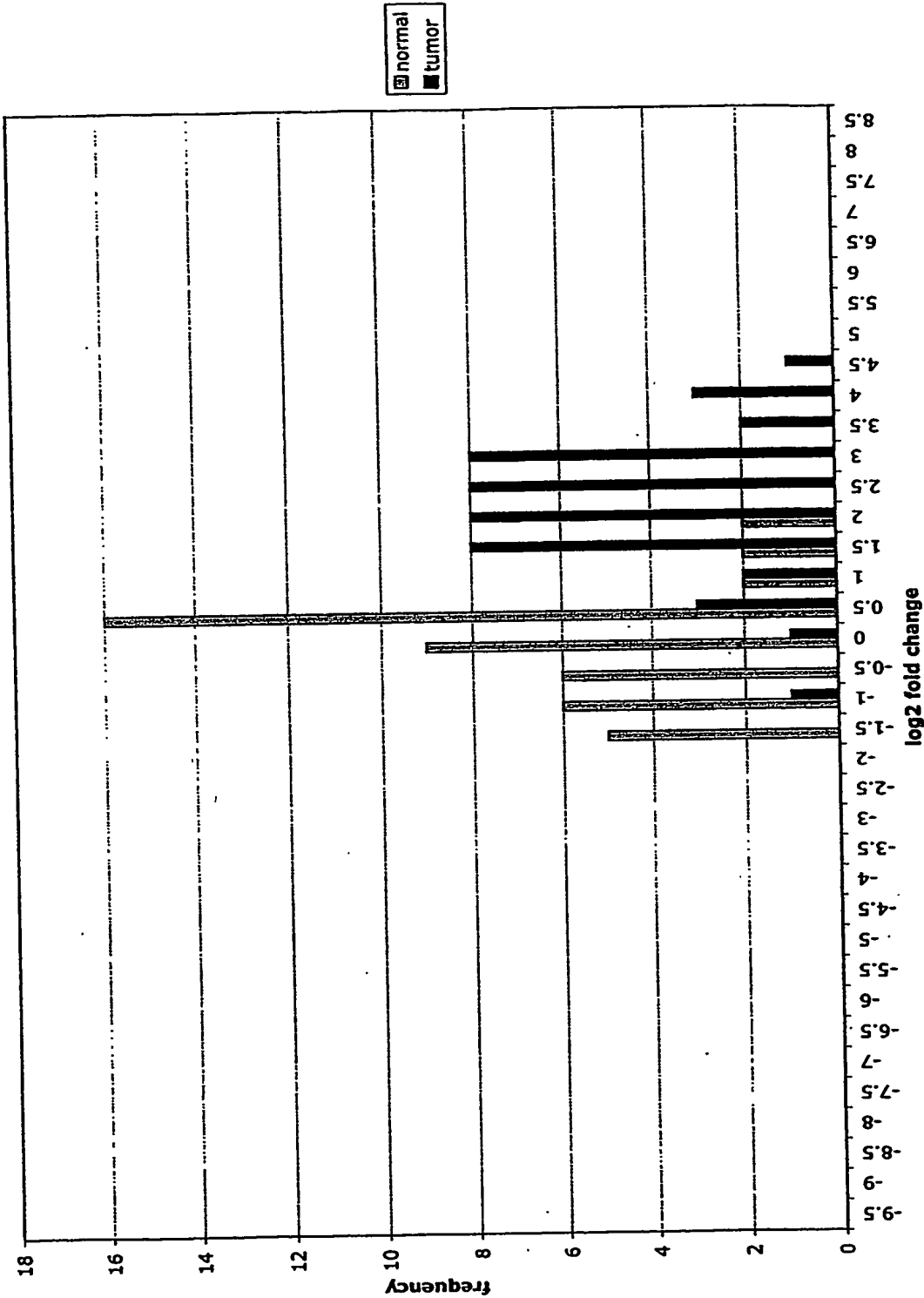


Figure 5(d)

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INHBA-tumor:median normal log2 fold change

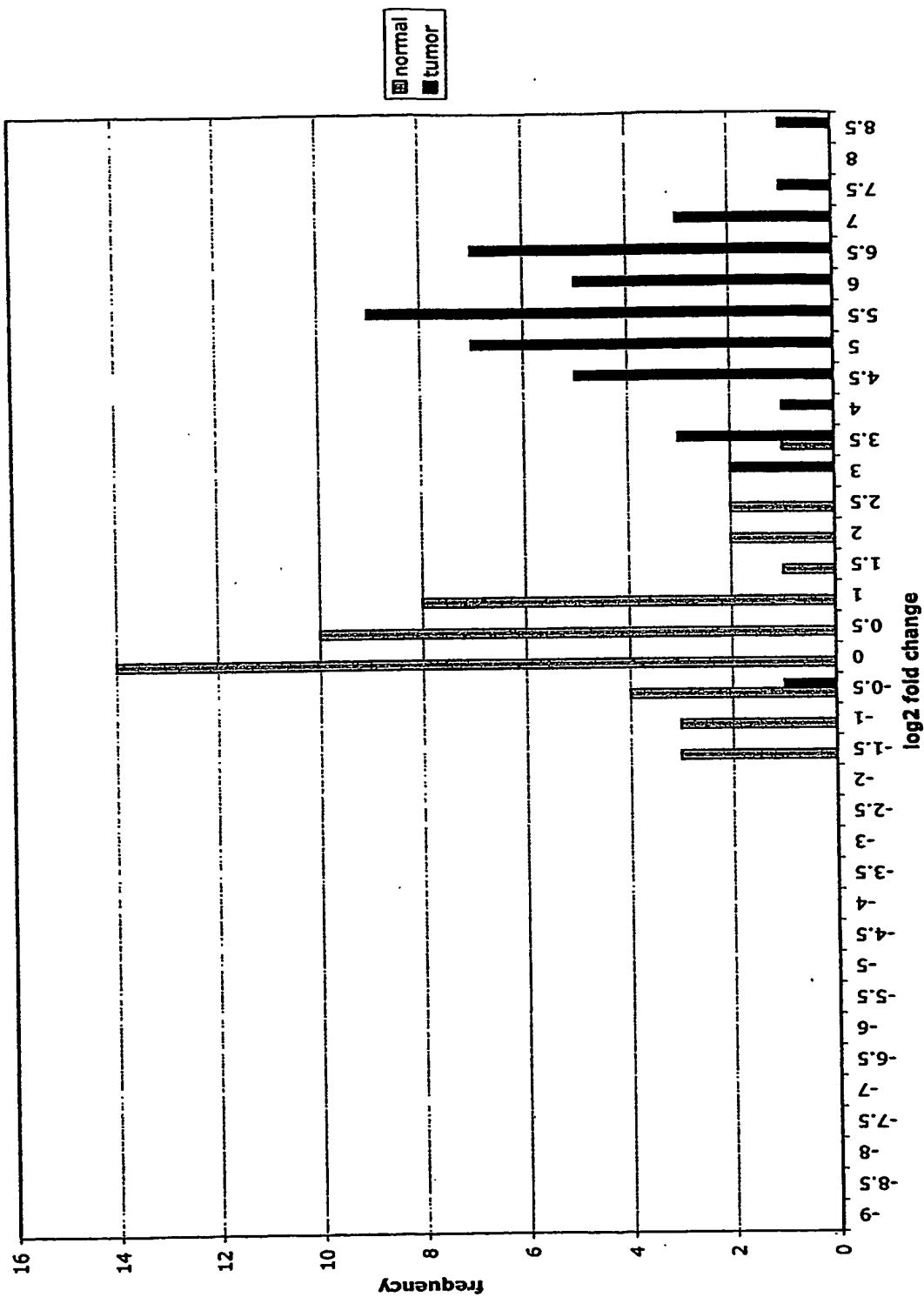


Figure 5(e)

LOXL2-tumor:median normal log2 fold changes

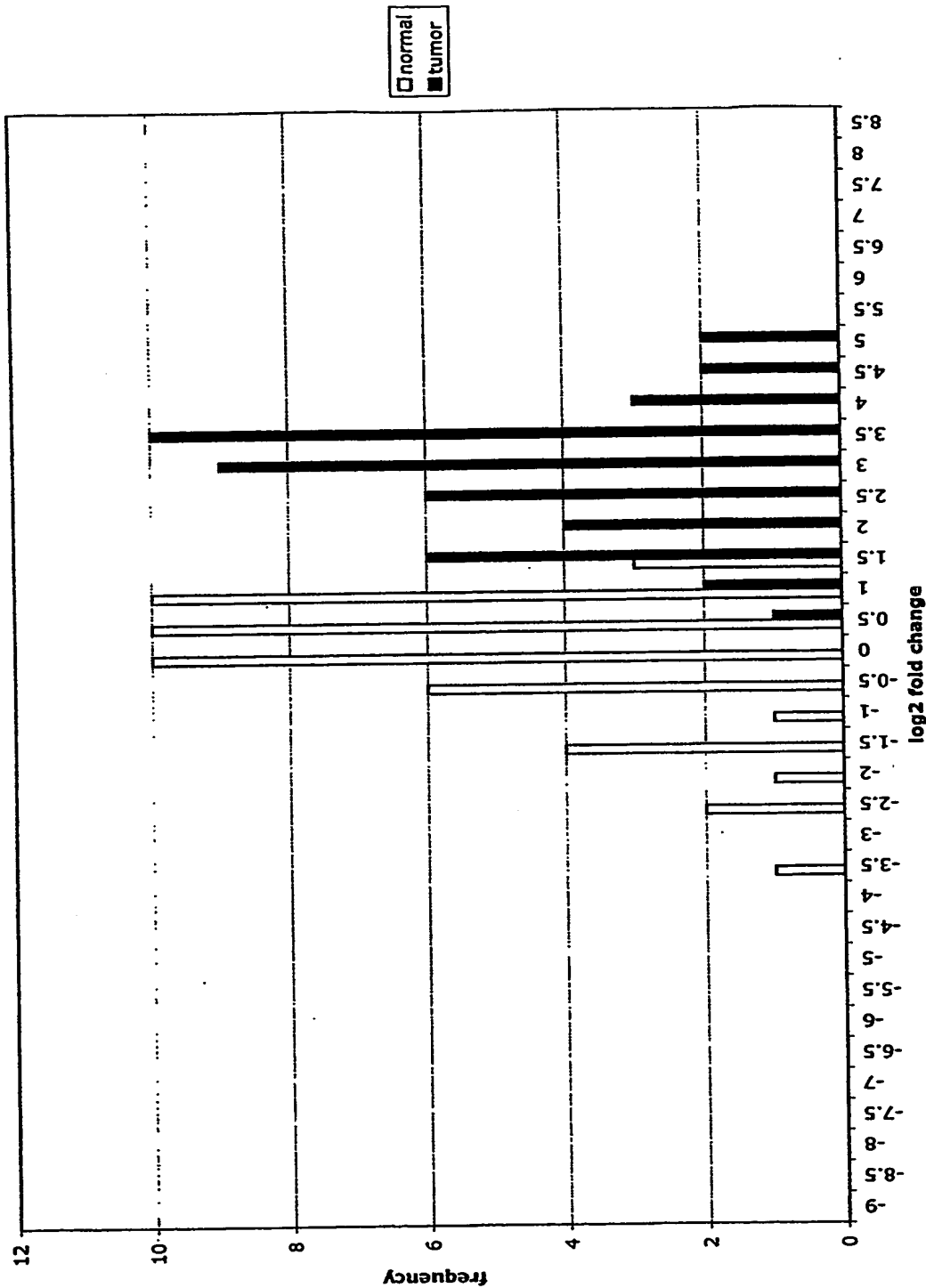


Figure 5(f)

lumican-Tumor:median normal log2 fold changes

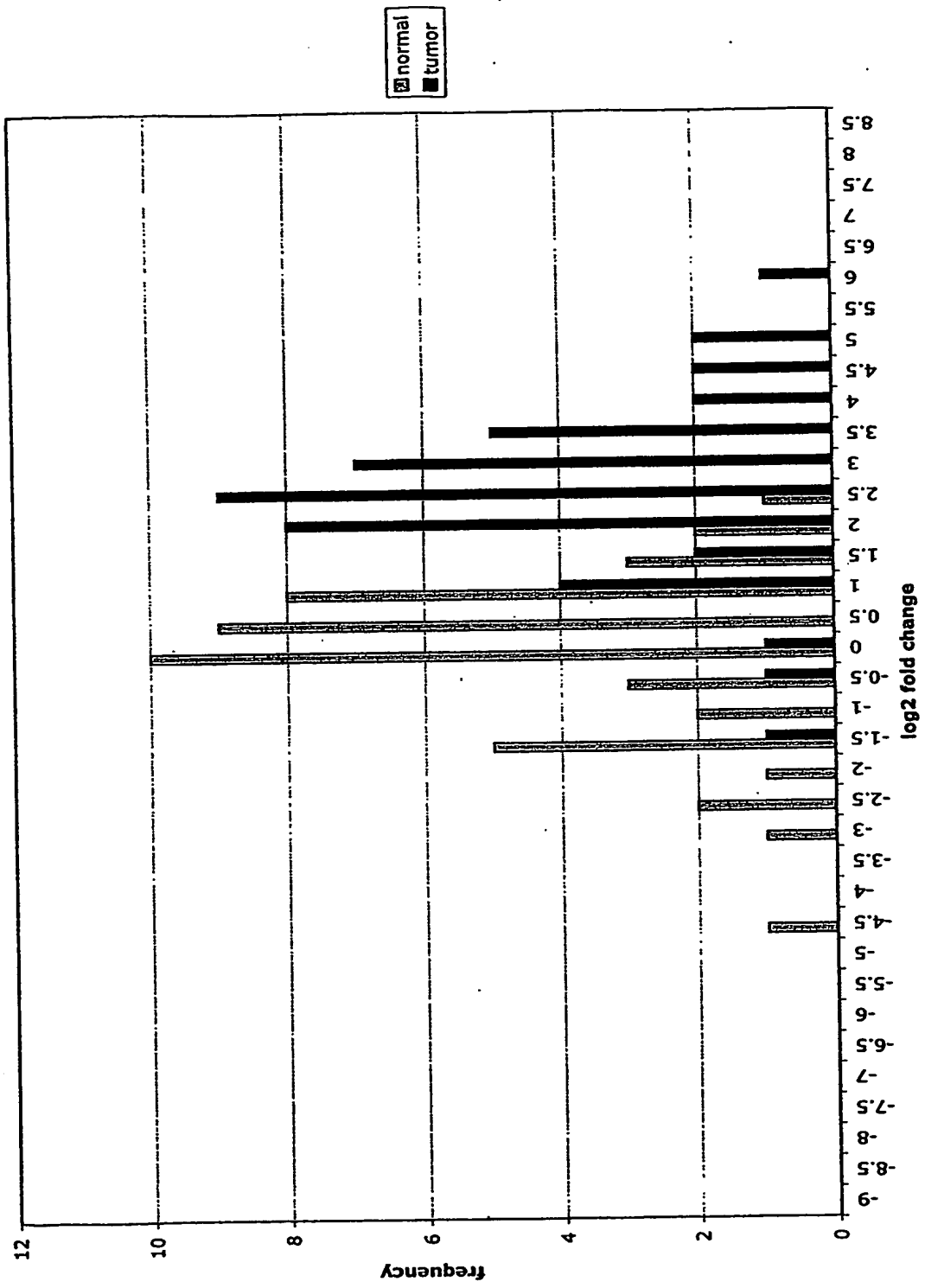


Figure 5(g)

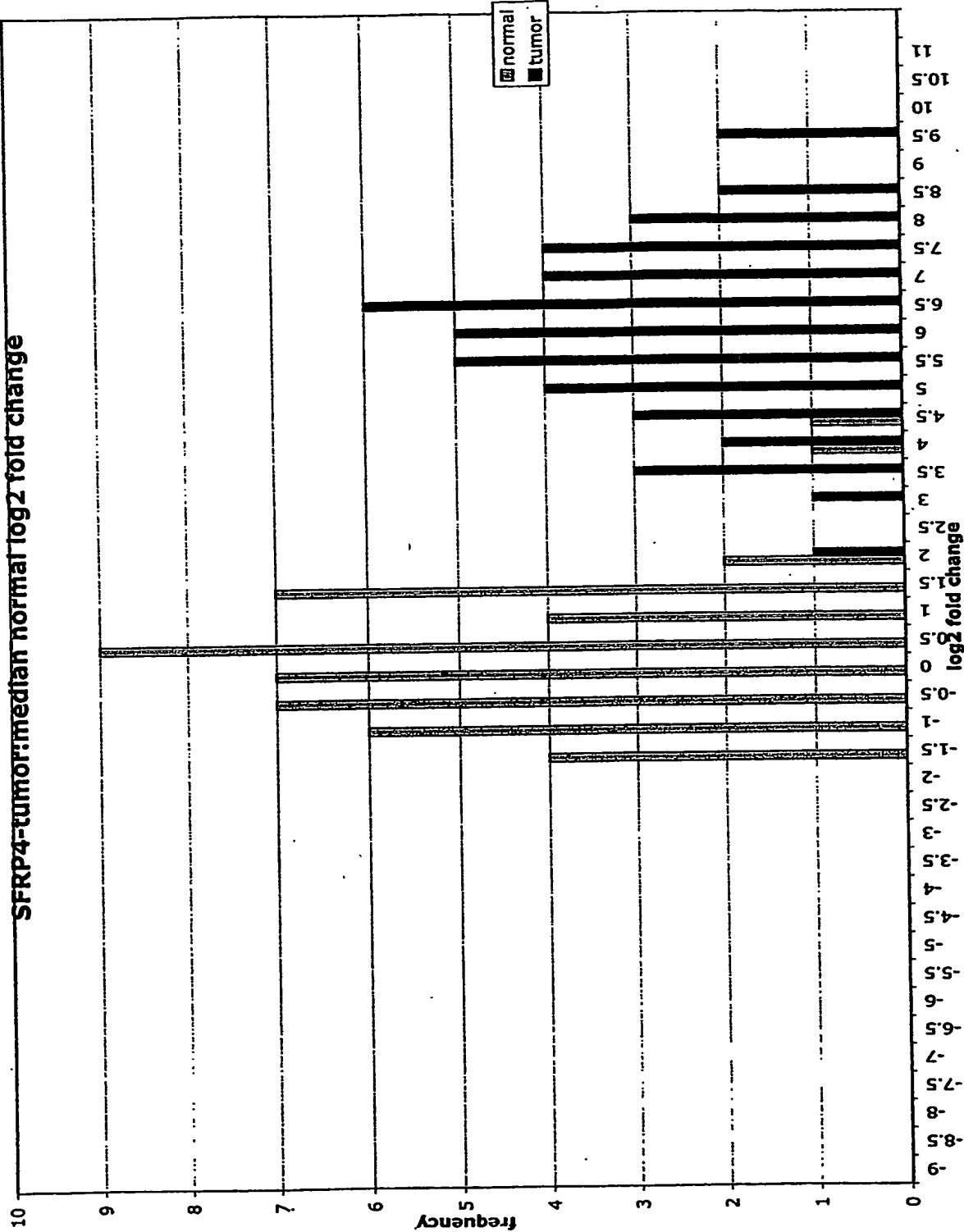


Figure 5(h)

SPARC-tumor:median normal log2 fold changes

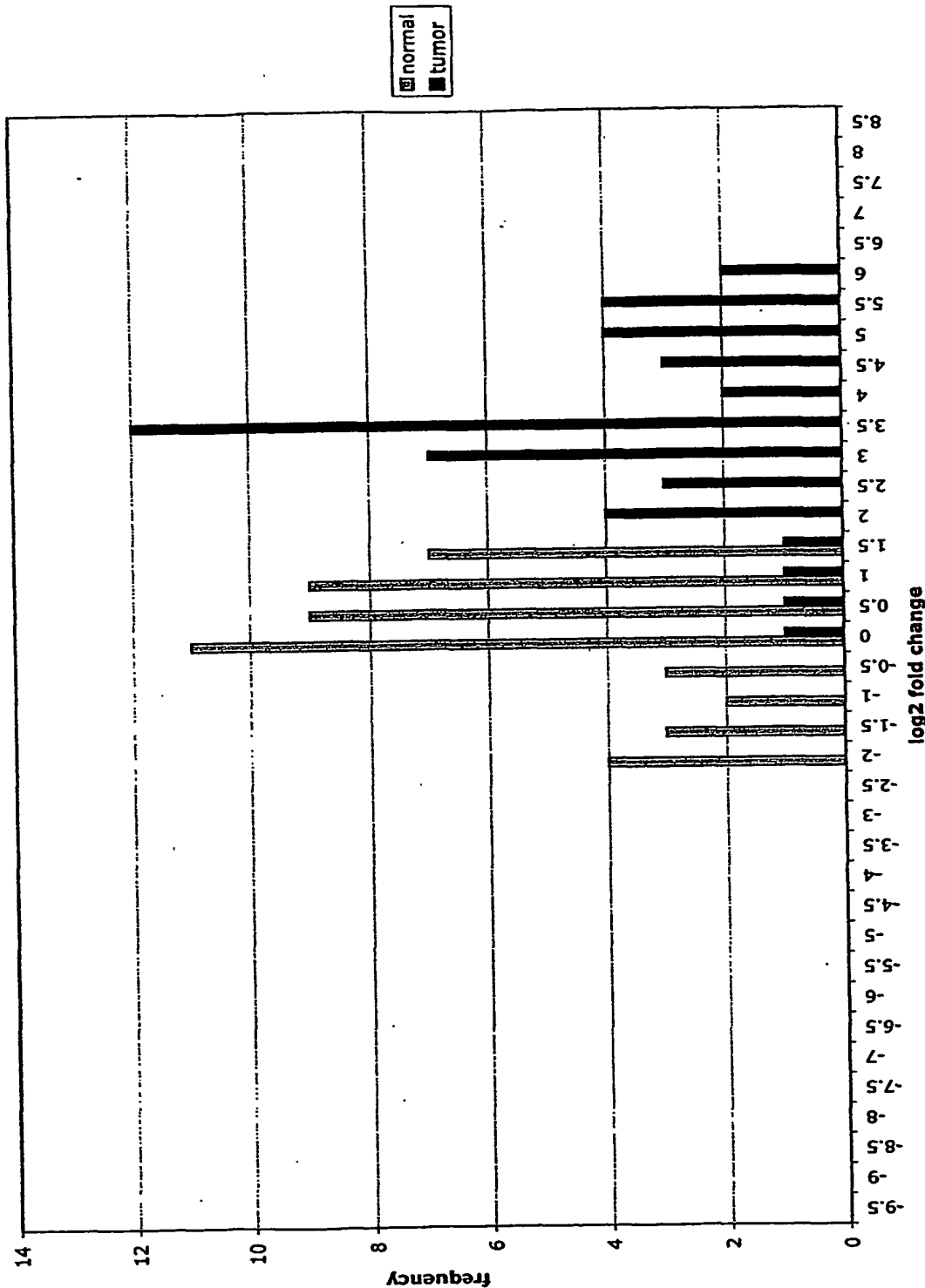


Figure 5(i)



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SPP1-tumor:median normal log2 fold change

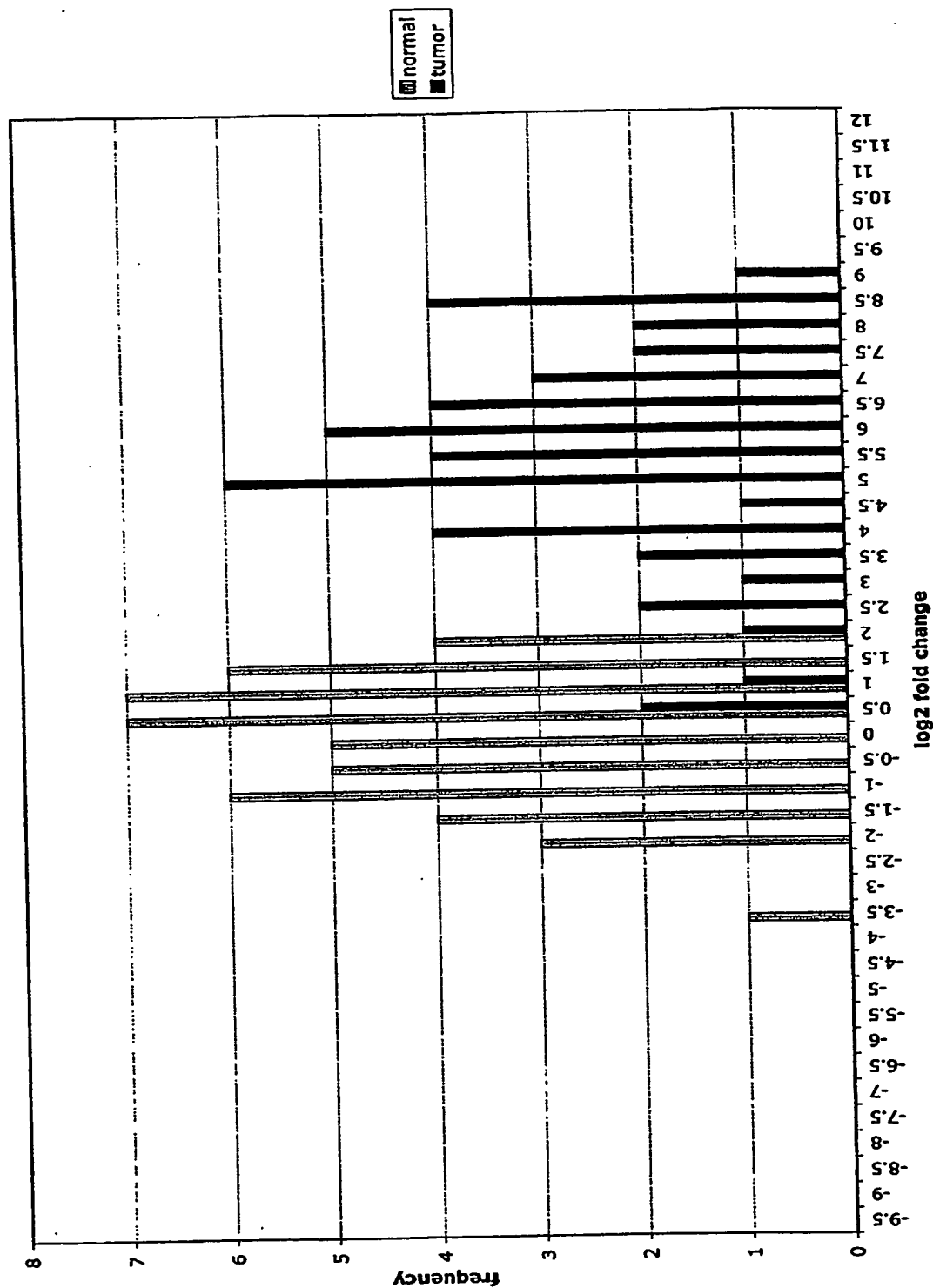


Figure 5(j)

THBS2-tumor:median normal log2 fold change

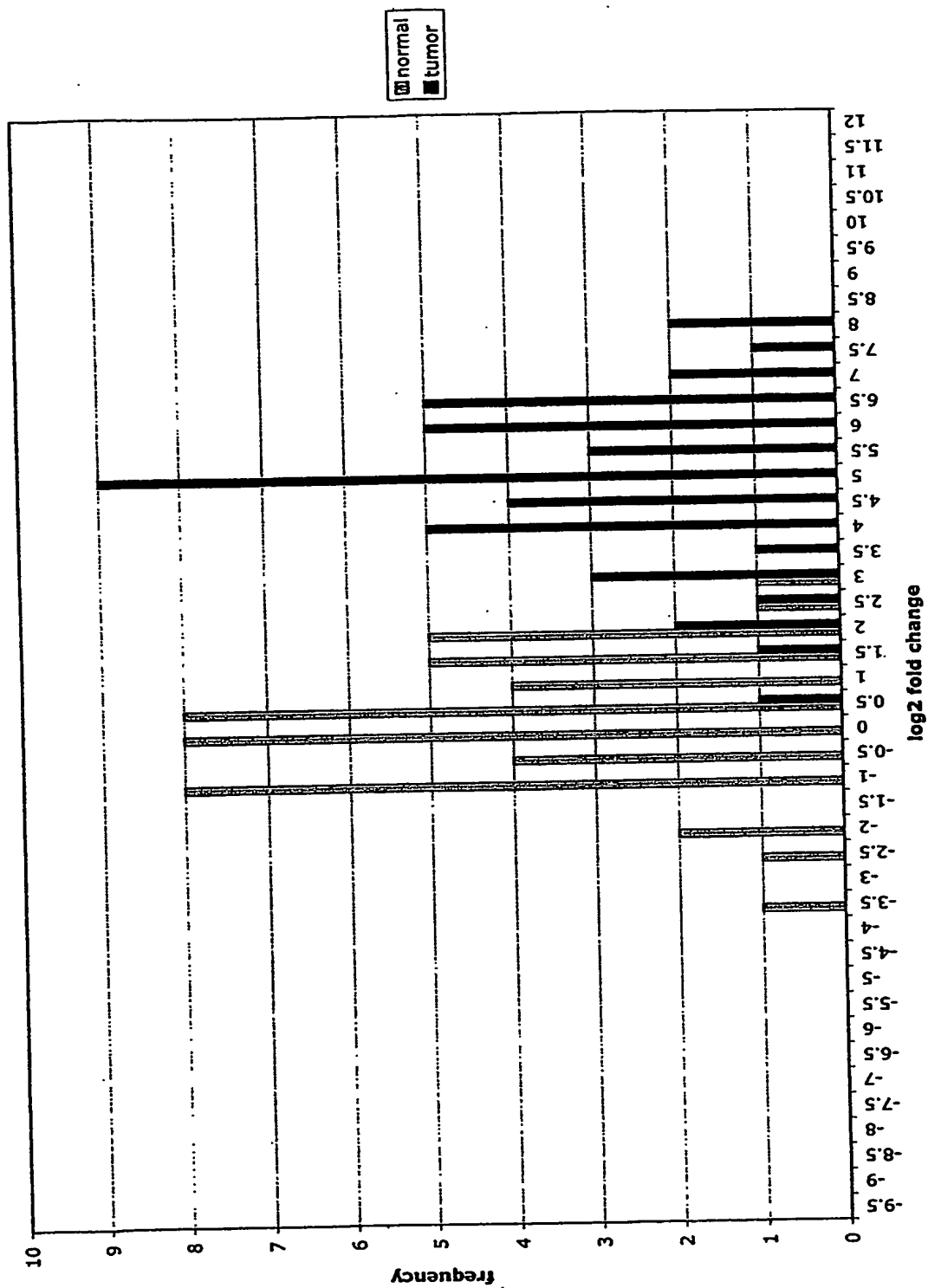


Figure 5(k)

TIMP1-tumor:median normal log2 fold change

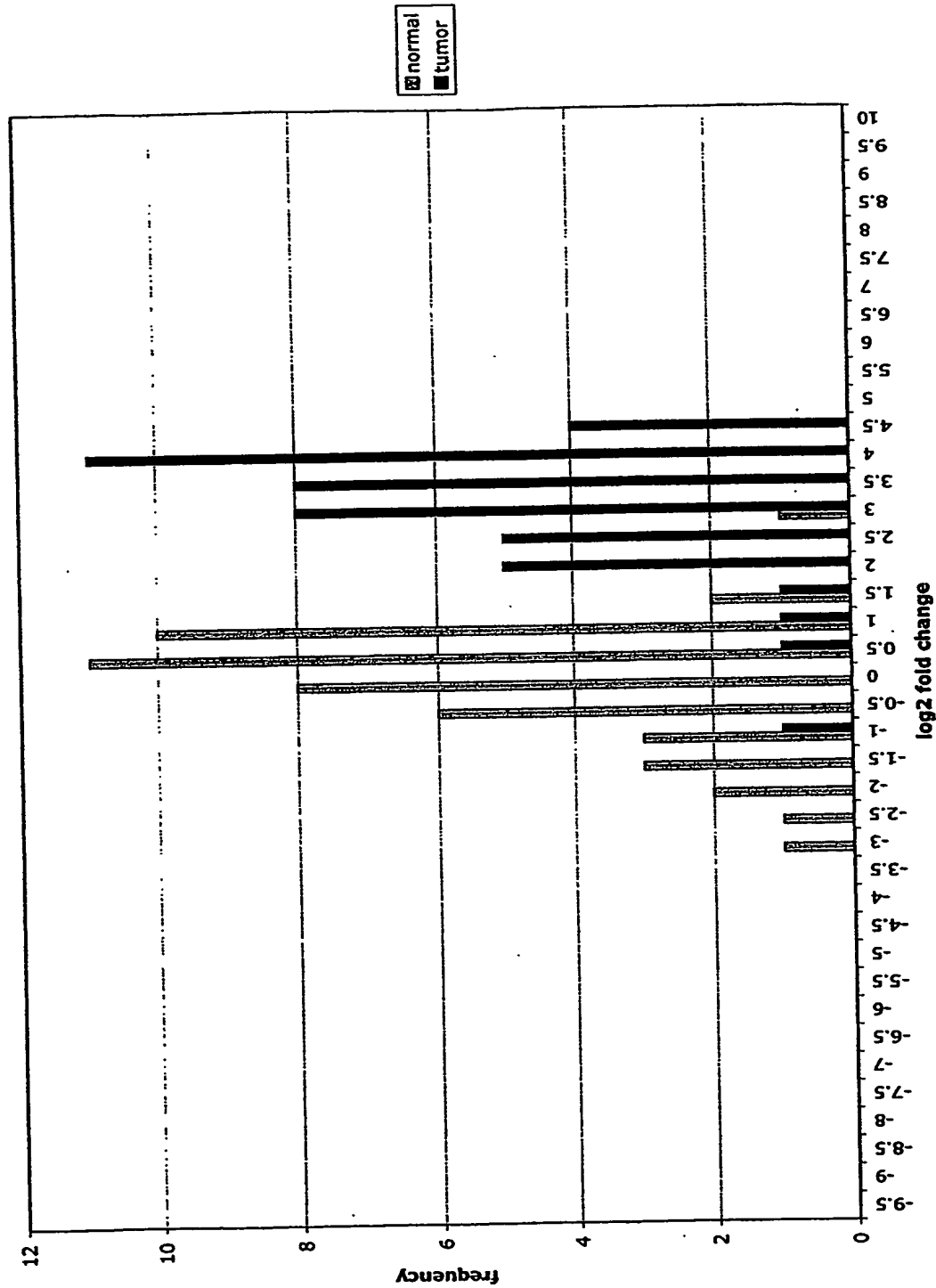


Figure 5(l)

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adlcan-tumor:median normal log2 fold change

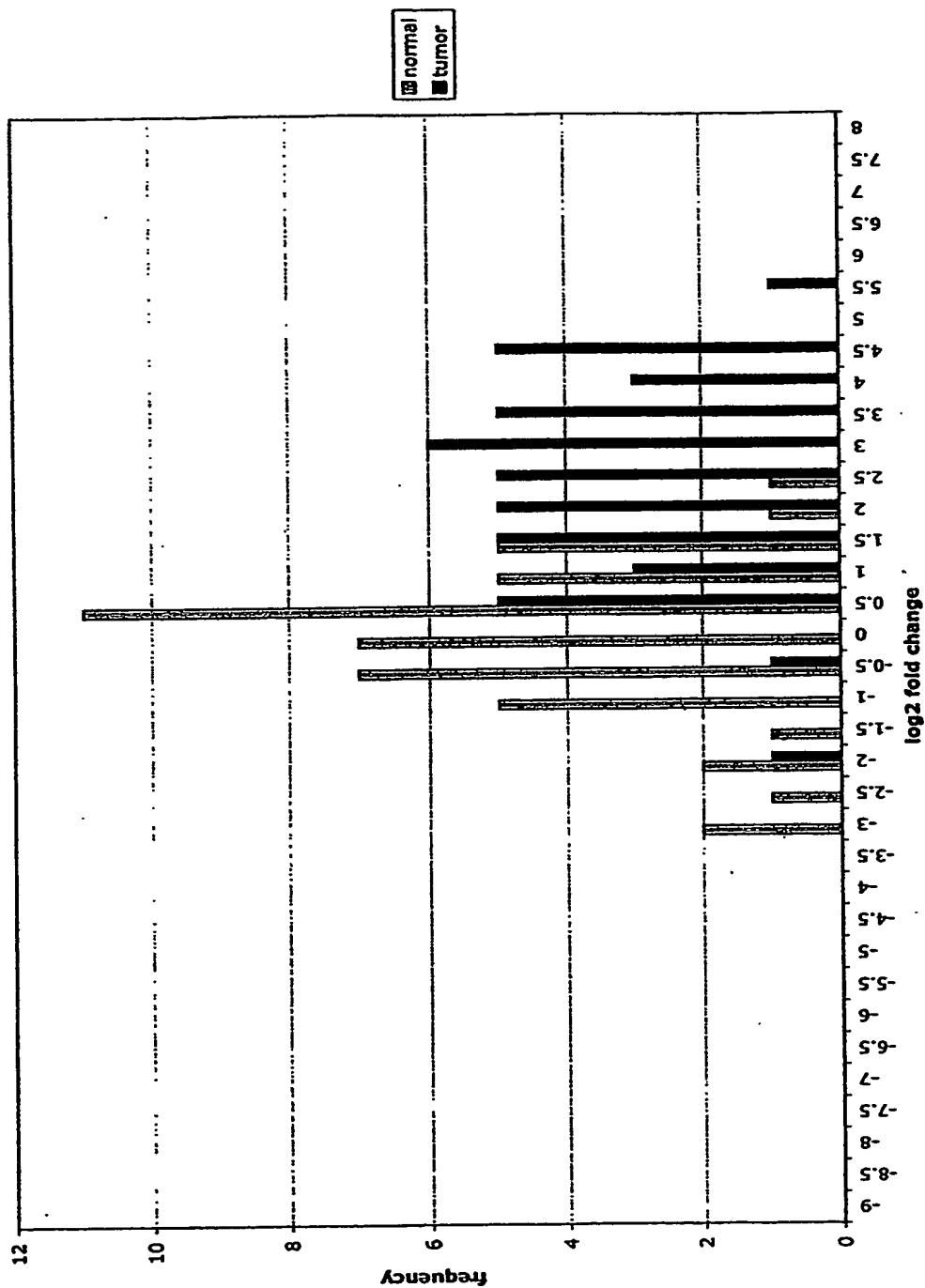


Figure 5(m)

PRS11- tumor:median normal log2 fold change

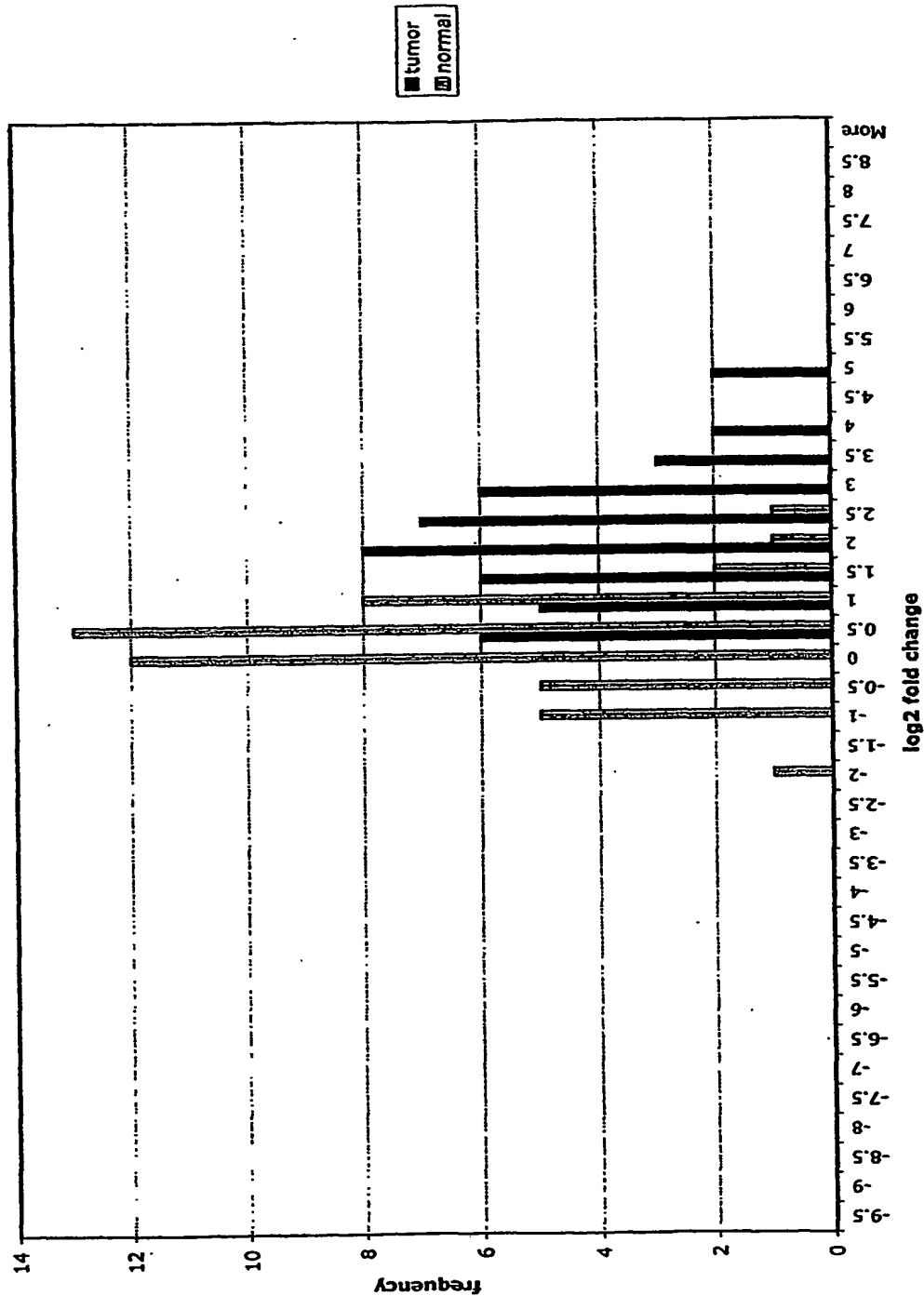


Figure 5(n)

ASAH1-tumor:median normal log2 fold changes

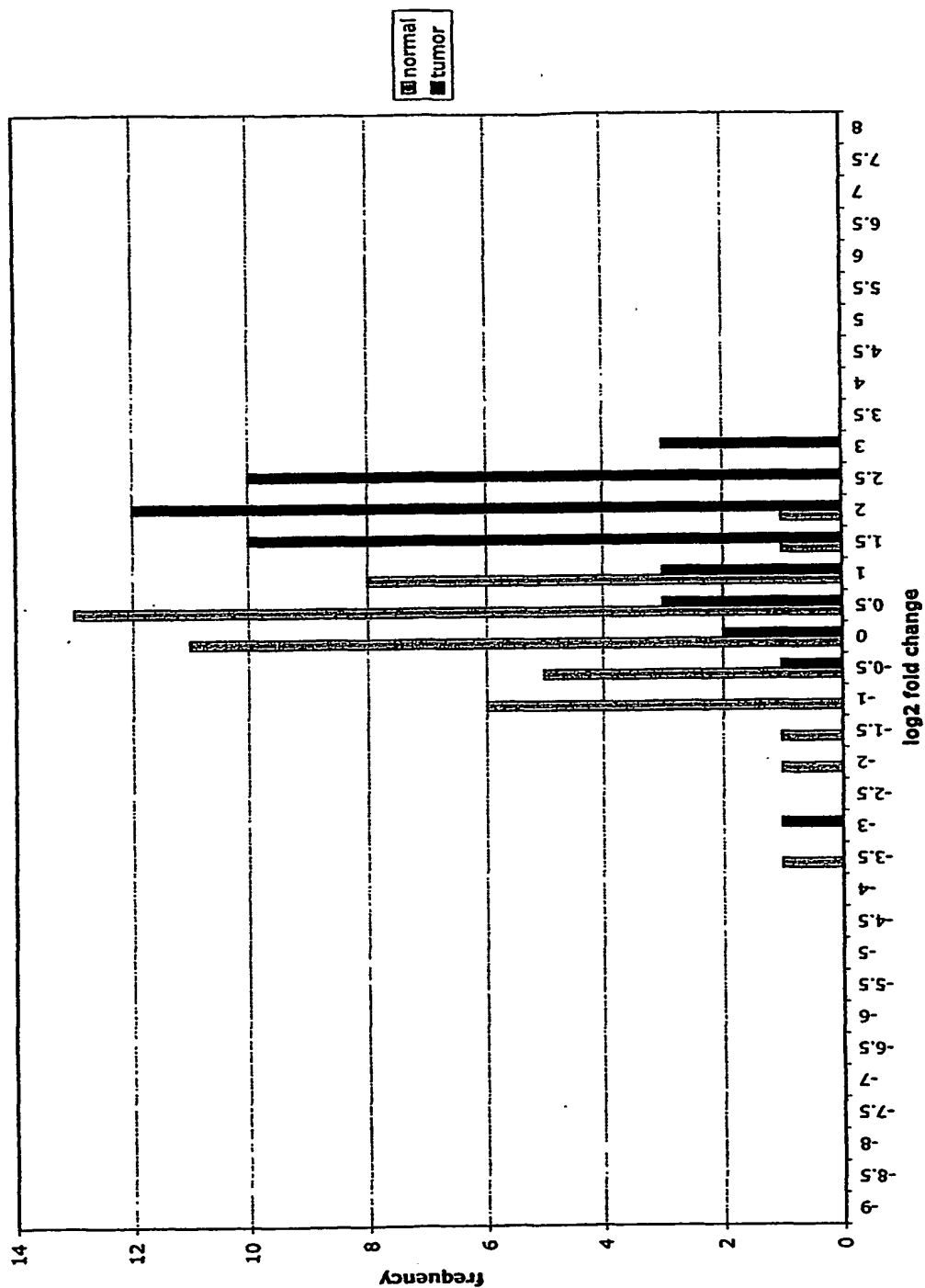


Figure 5(o)

SFRP2-tumor:median normal log2 fold change

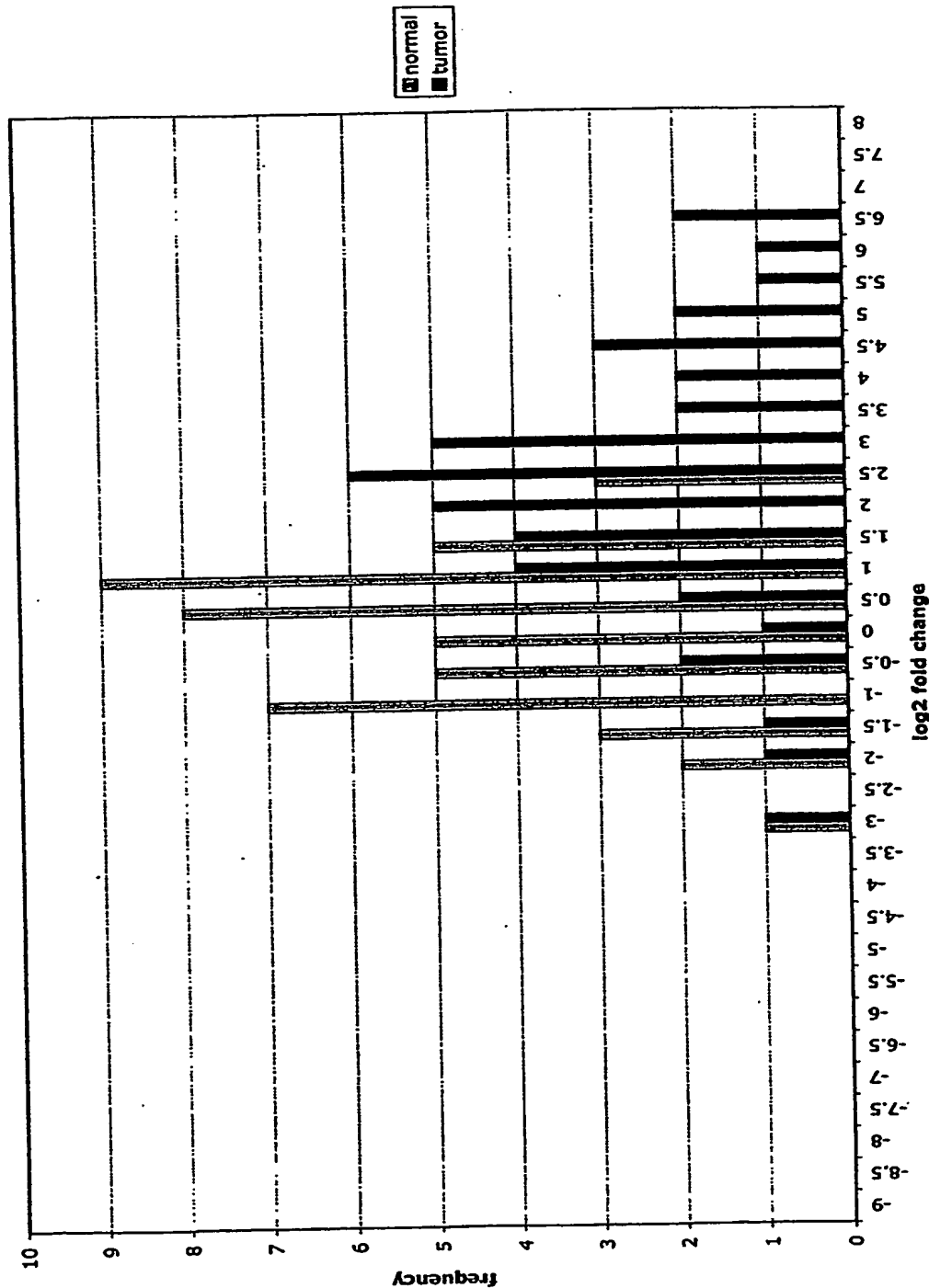


Figure 5(p)

GGH-tumor:normal log2 fold change

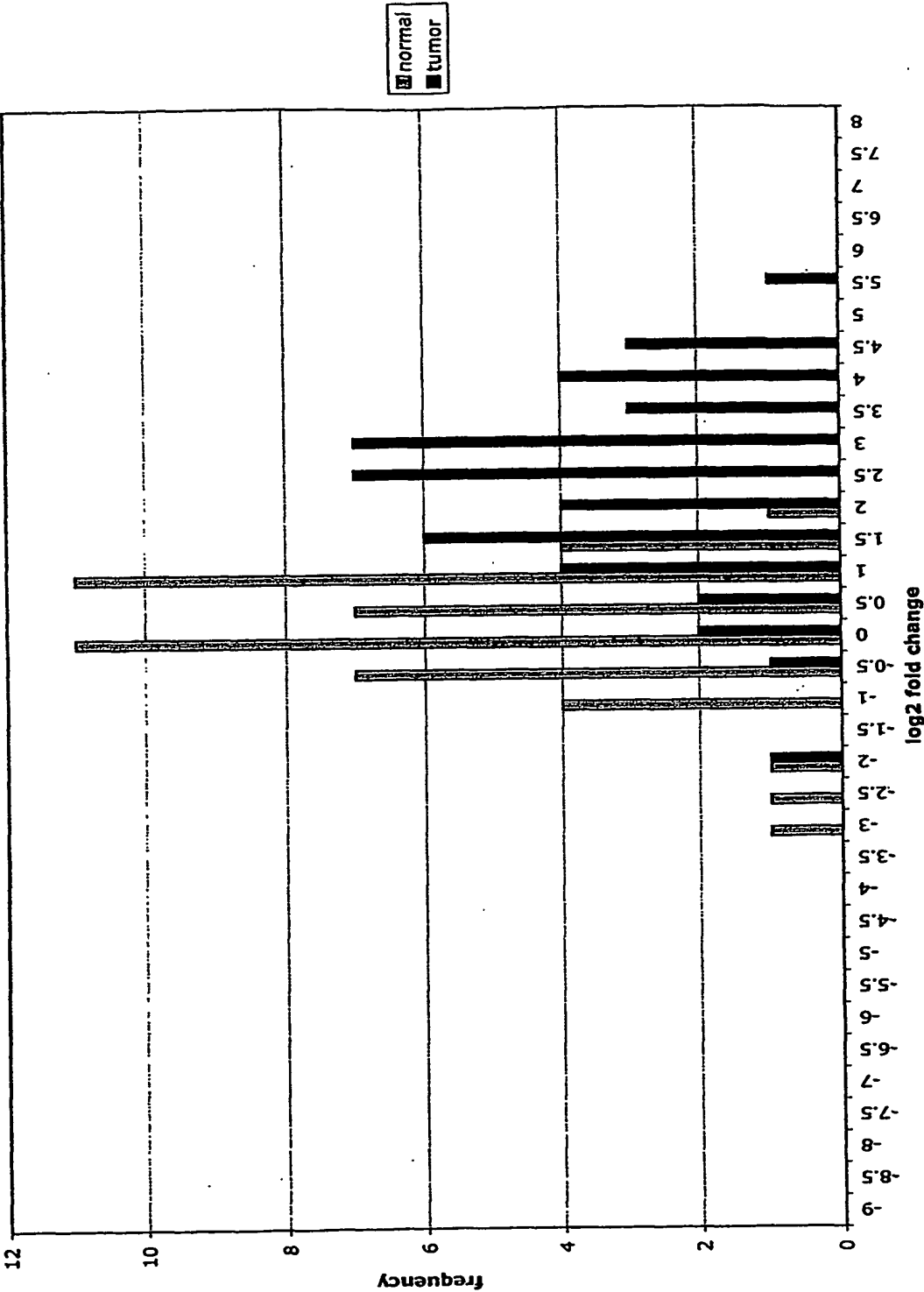


Figure 5(q)



MMP12-tumor:median normal log2 fold changes

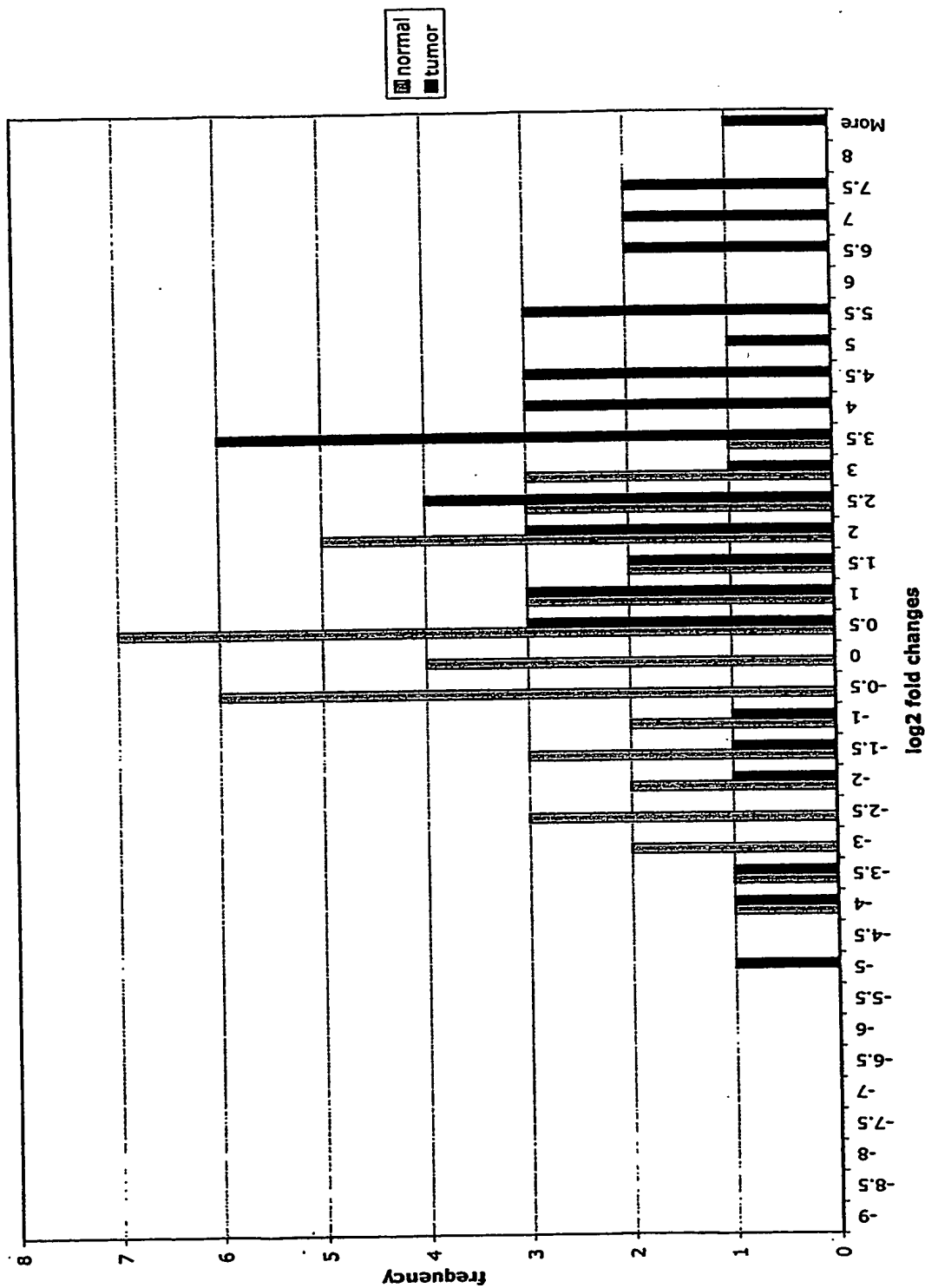


Figure 5(r)

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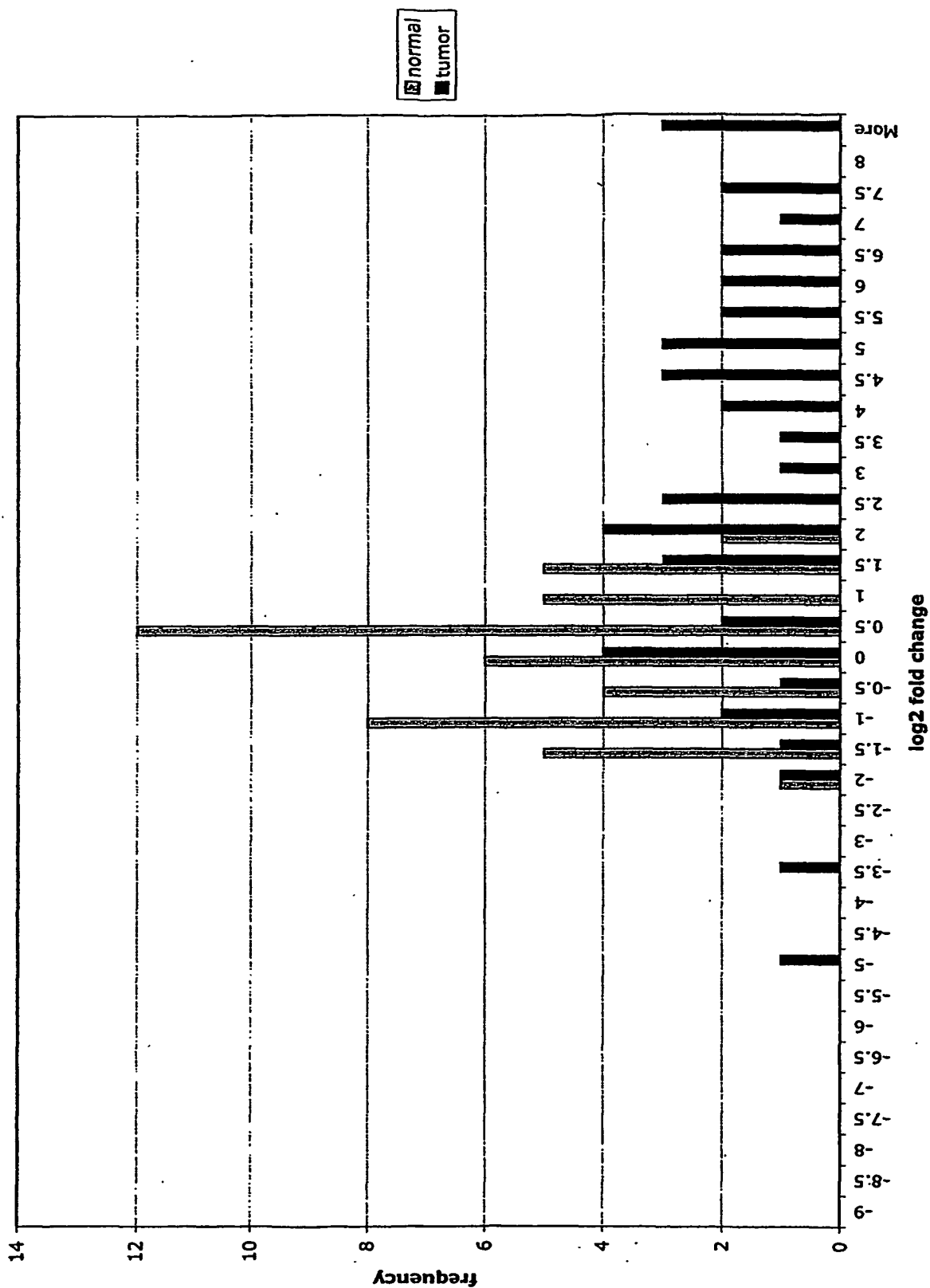


Figure 5(s)

LEPRE1-tumor:median normal log2 fold changes

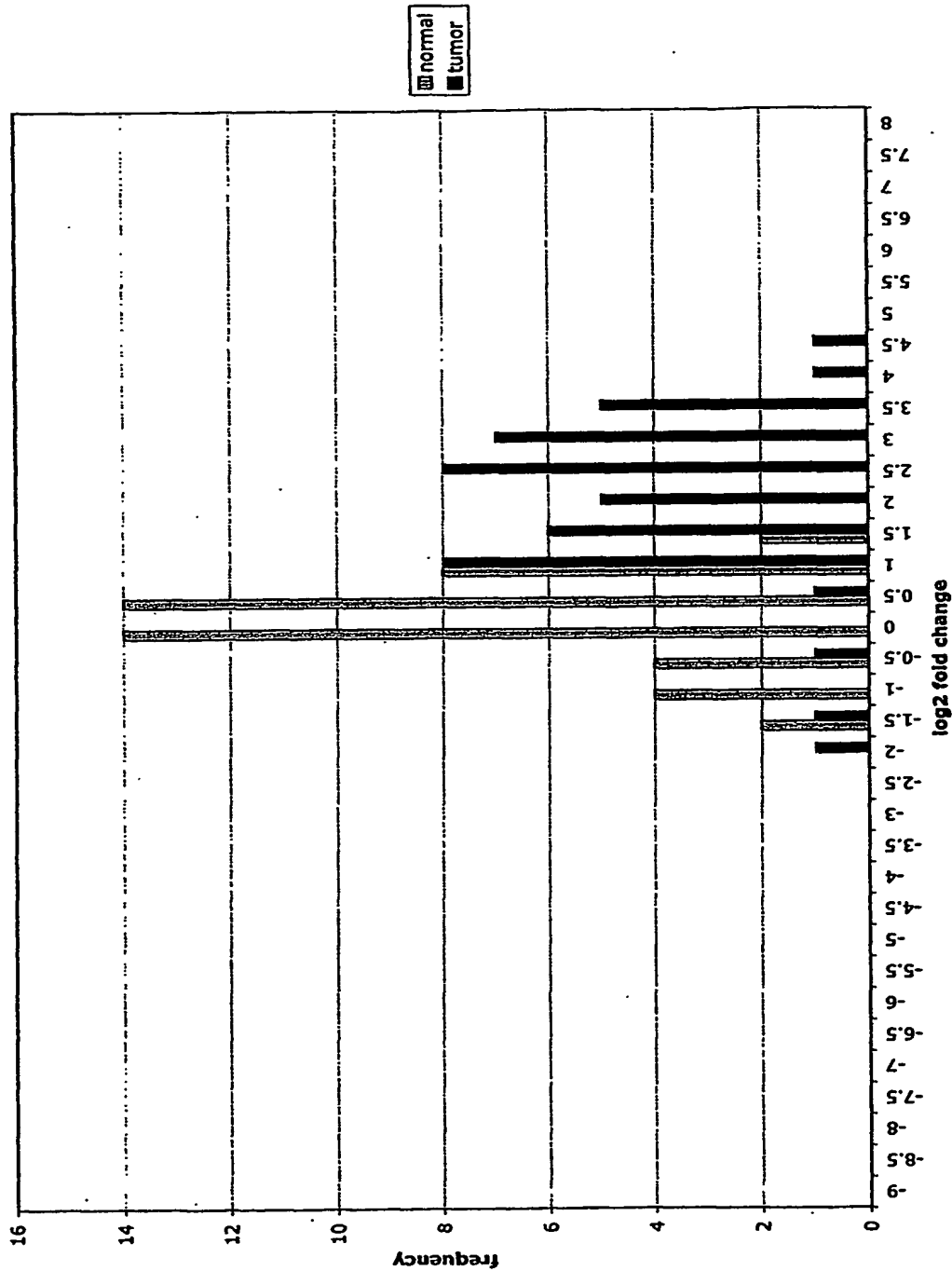


Figure 5(t)

TG-tumor:median normal log2 fold change

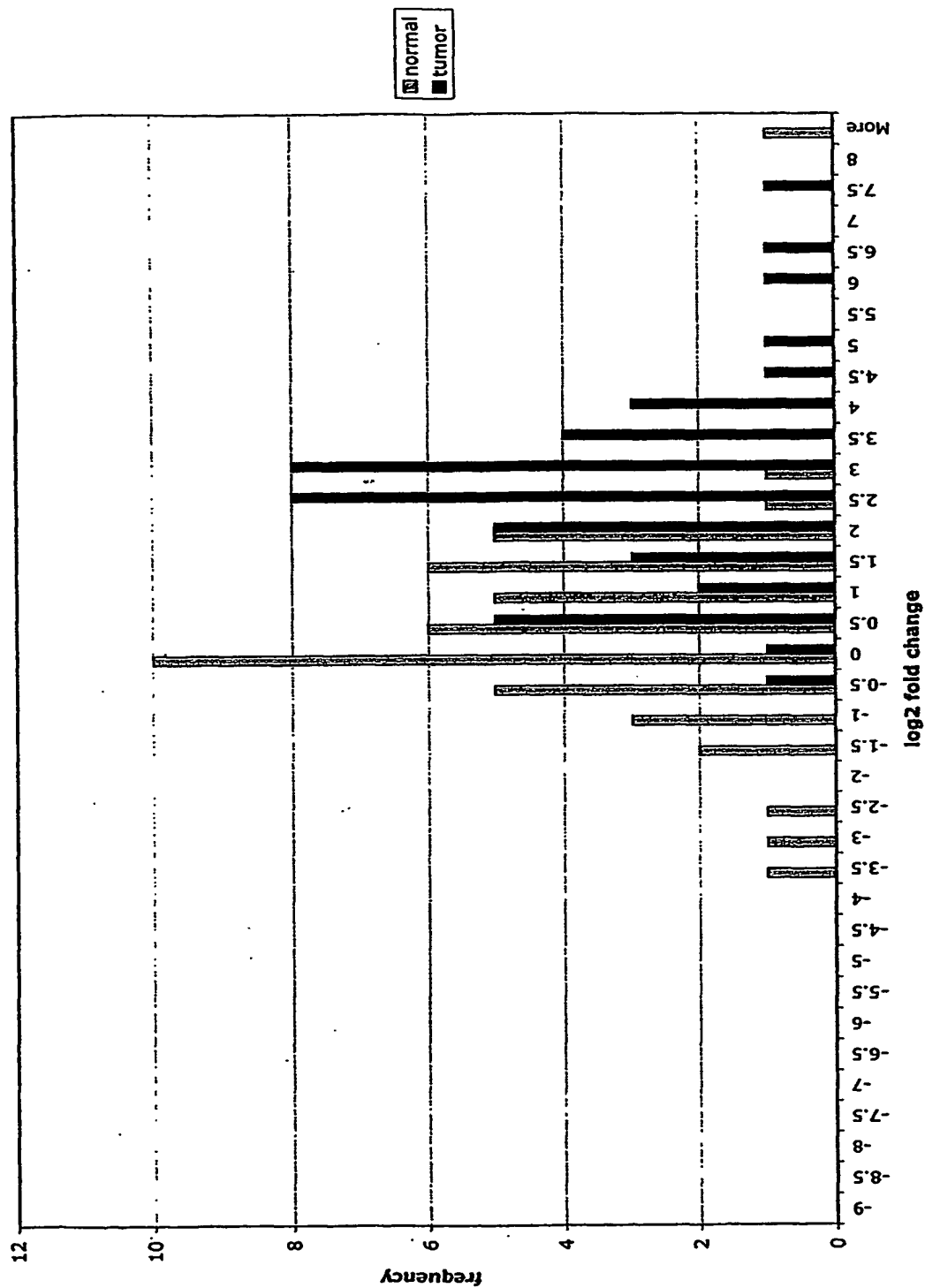


Figure 5(u)

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EFEMP2-tumor:median normal log2 fold change

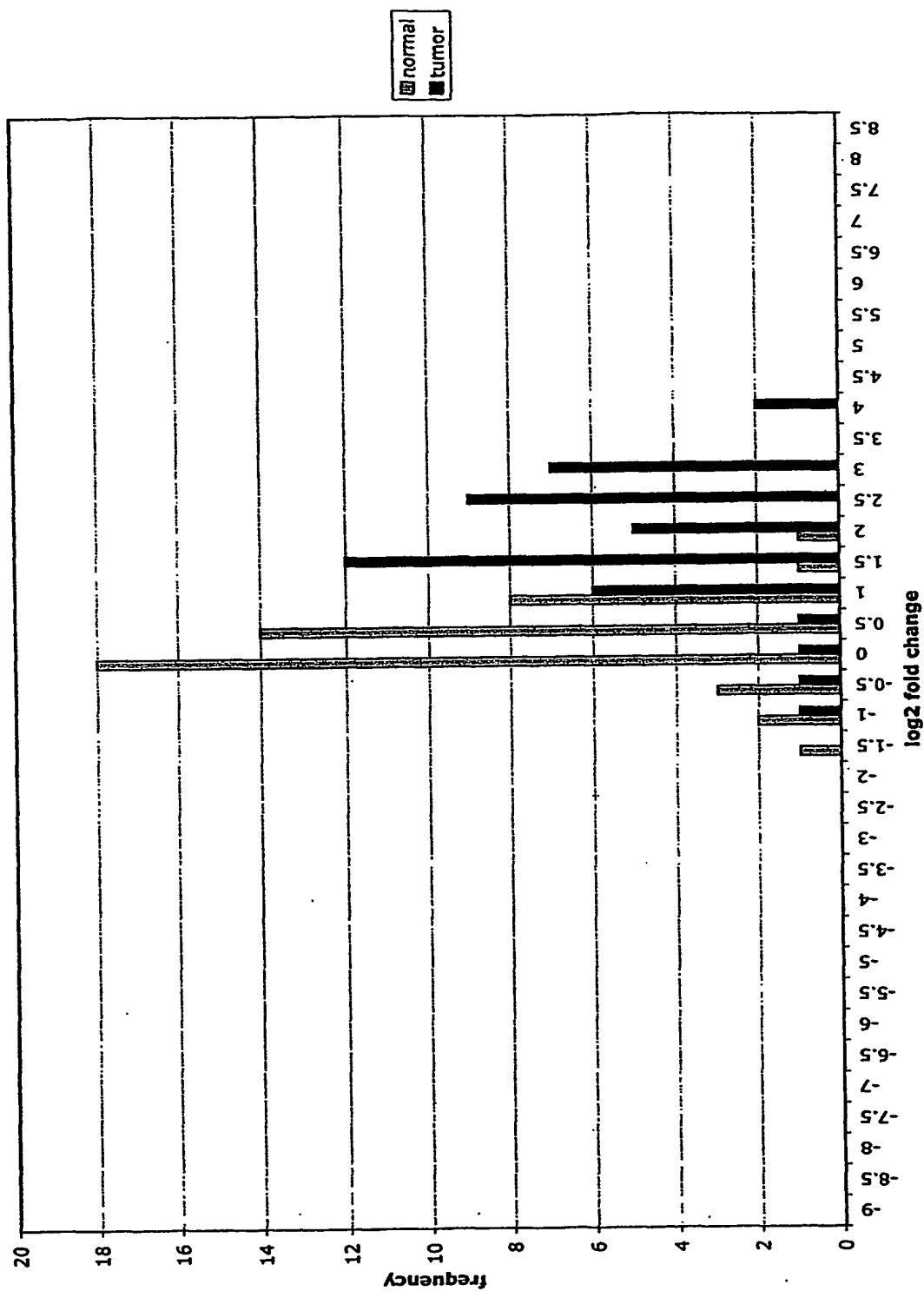


Figure 5(v)

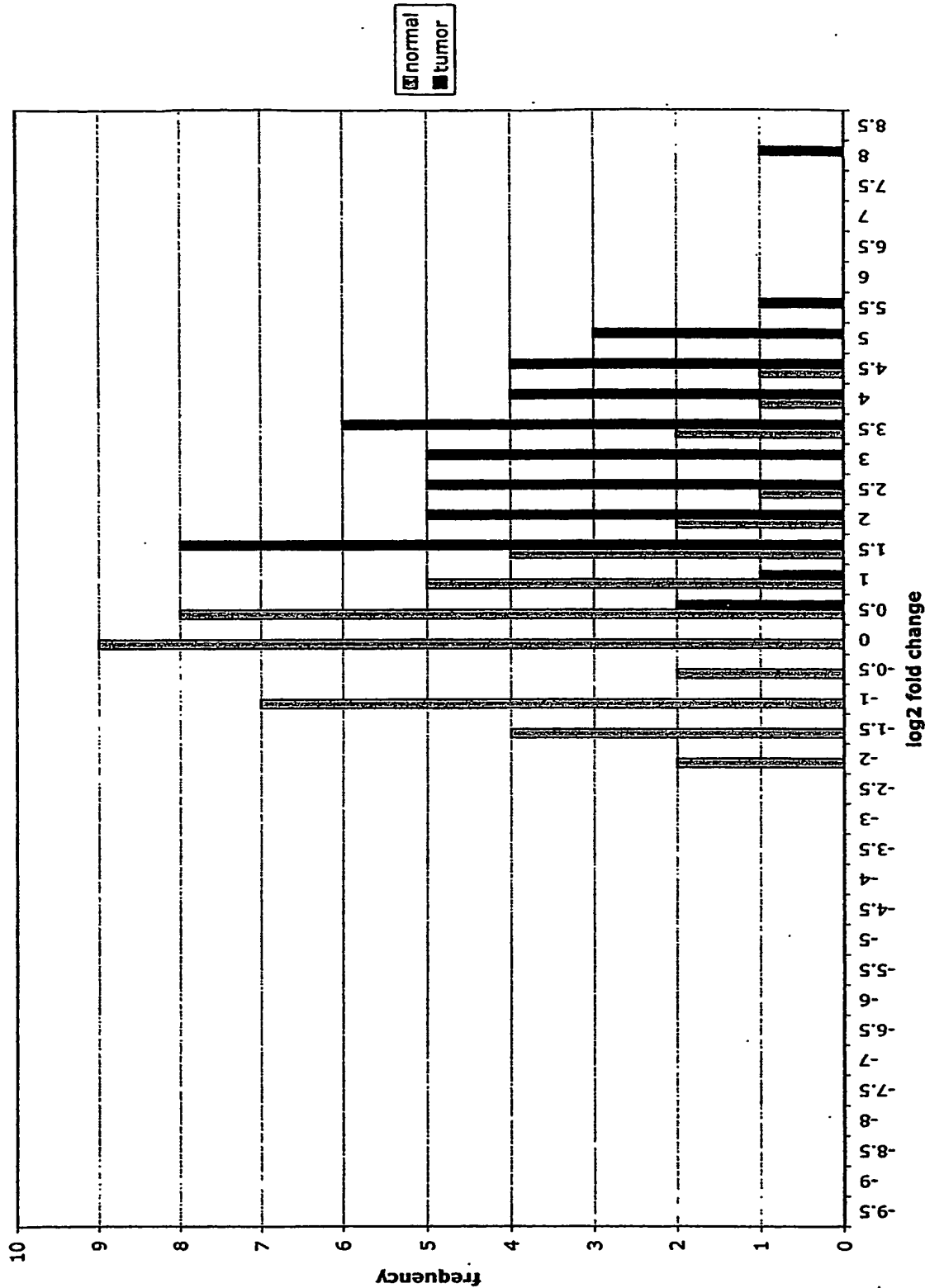


Figure 5(w)

Number of genes expressed > 95th percentile of median normal expression in each tumor sample

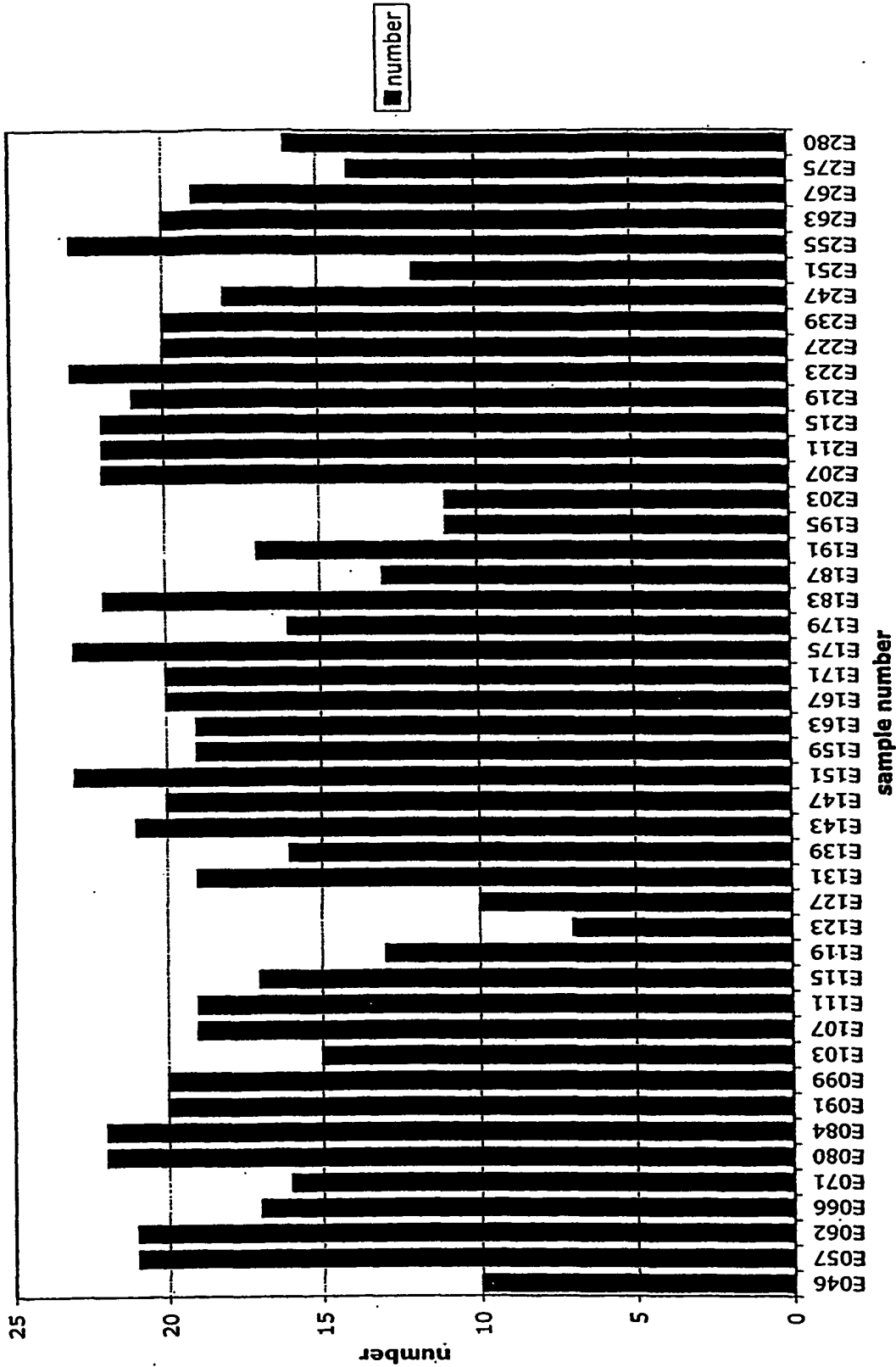


Figure 6

**Fig.7a** Relative expression of markers in tumor and normal samples compared to CEA

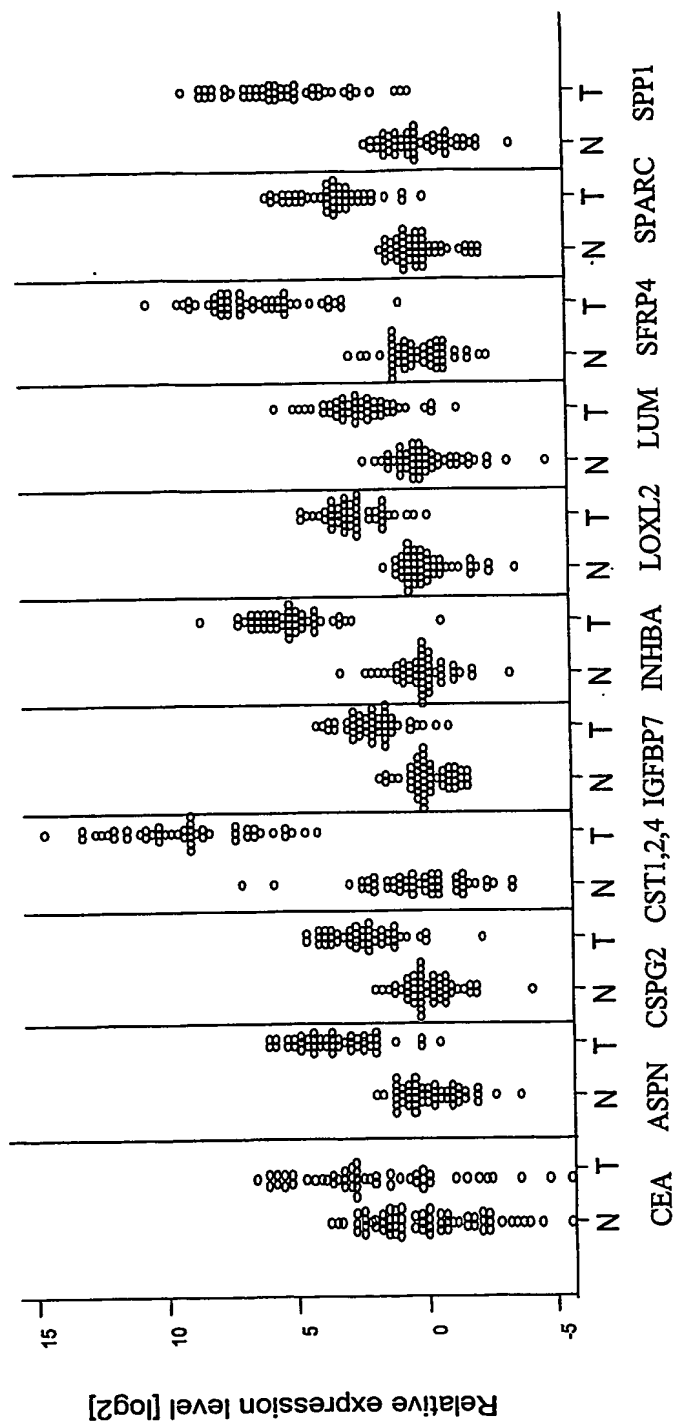




Fig. 7b

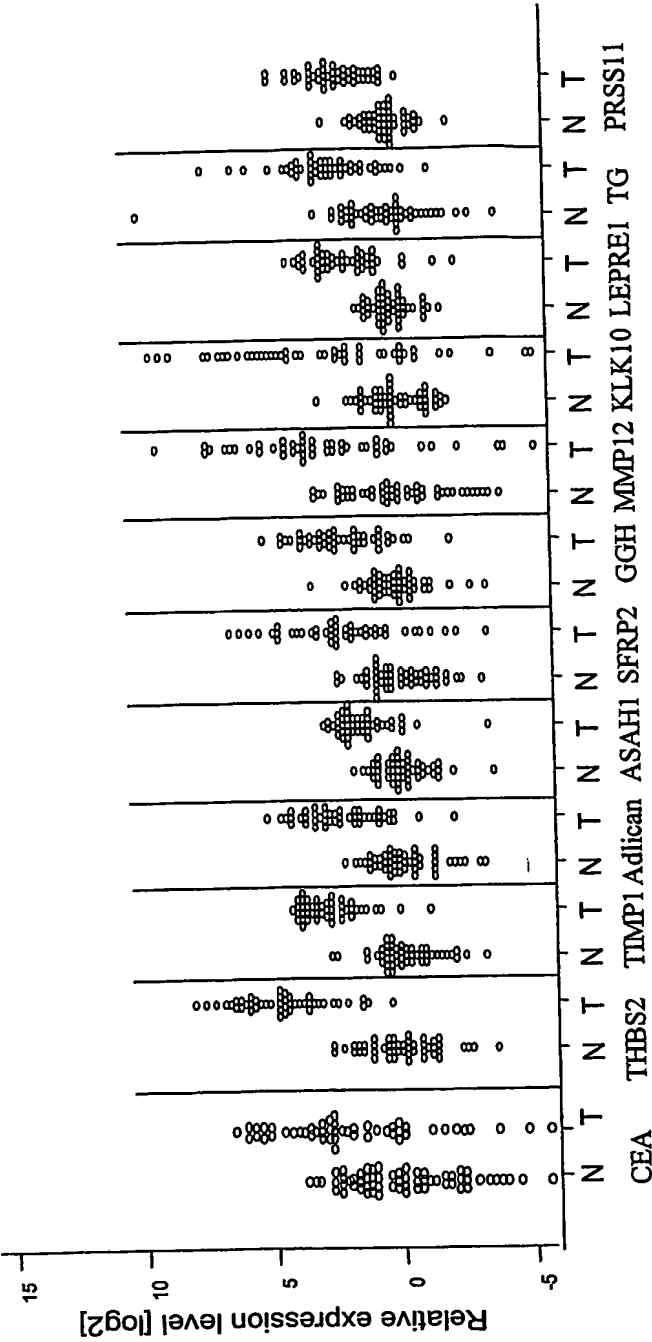


Fig. 7c

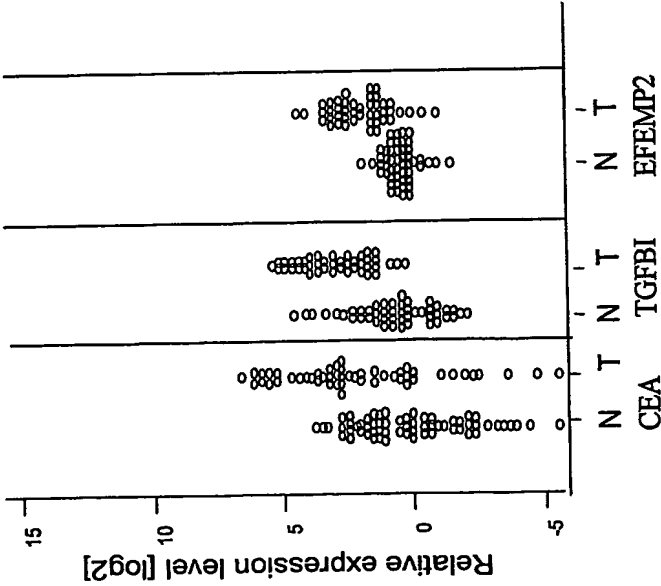


Fig. 8. Quantitative RT-PCR: expression in paired tumor and non-malignant samples of selected gastric cancer markers					
name	symbol	median T:N fold change	maximum T:N fold change	% tumor samples with expression > paired non-malignant sample	
adipon		5	146	88	
asporin (irr class 1)	ASPN	11	198	100	
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	5	68	93	
cystatins SN, SA & S	CST1, 2, 4	498	11911	100	
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	3	17	93	
gamma-glutamyl hydrolase	GGH	4	34	83	
inhibin beta A chain	INHBA	27	630	95	
insulin-like growth factor binding protein 7	IGFBP7	5	38	93	
kallikrein 10	KLK10	7	519	78	
leudine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1	4	23	85	
lumican	LUM	5	68	90	
lysyl oxidase-like 2	LOXL2	7	53	95	
matrix metalloproteinase 12	MMP12	9	468	85	
metalloproteinase inhibitor 1	TIMP1	6	103	95	
n-acylsphingosine amidohydrolase	ASAH1	3	15	88	
osteopontin	SPP1	36	626	98	
secreted frizzled-related protein 2	SFRP2	5	48	83	
secreted frizzled-related protein 4	SFRP4	54	375	100	
secreted protein, acidic, cysteine rich	SPARC	10	66	95	
serine protease 11 (IGF binding)	PRSS11	4	63	90	
thrombospondin 2	THBS2	23	452	98	
thyroglobulin	TG	4	174	93	
transforming growth factor B-induced	TGFB1	5	78	95	
cell growth regulatory factor with EF-hand domain	CGR11	3	33	75	
serine (or cysteine) proteinase inhibitor H1	SERPINH1	10	51	98	
matrix metalloproteinase 12	MMP2	2	46	83	
proprotein convertase subtilisin/kexin type 5	PCSK5	2	63	80	
serine (or cysteine) proteinase inhibitor B5	SERPINH5	5	861	73	
transforming growth factor beta	TGFB1	3	16	88	
cardiomyoblastic antigen (CEA)	CEACAM5	3	177	68	

**Fig. 9a** Relative tumor:normal fold changes in paired tumor/normal gastric samples

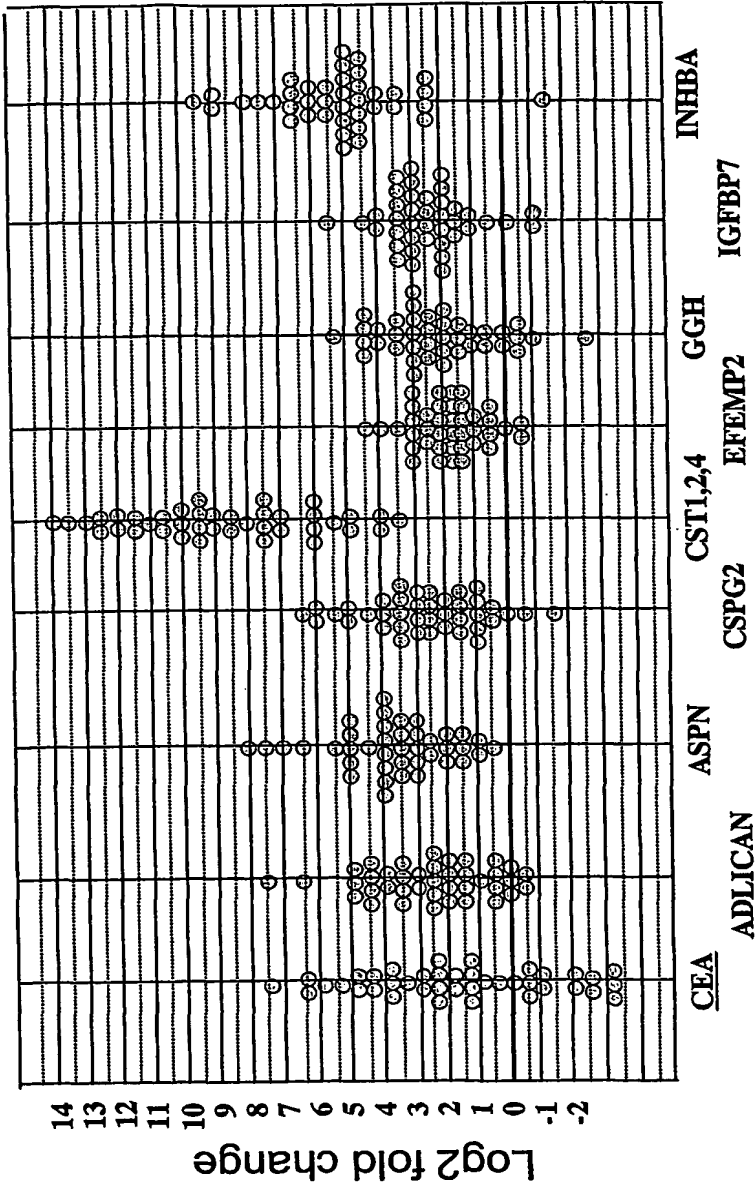


Fig. 9b

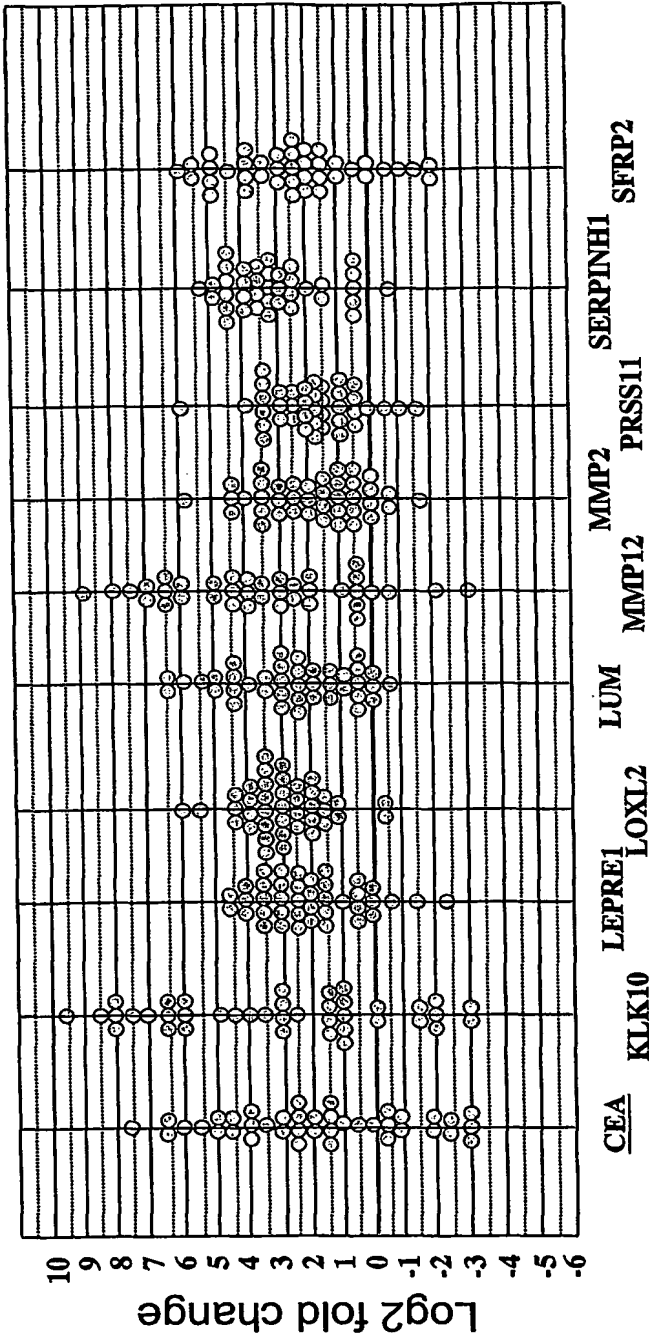


Fig. 9c

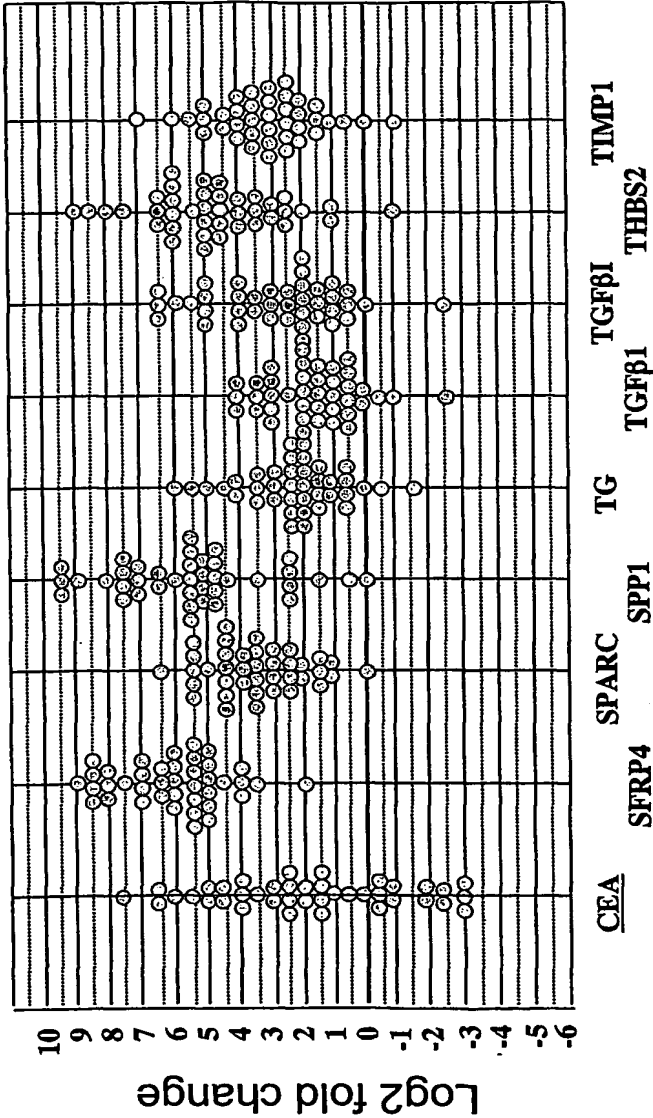


Fig. 9d

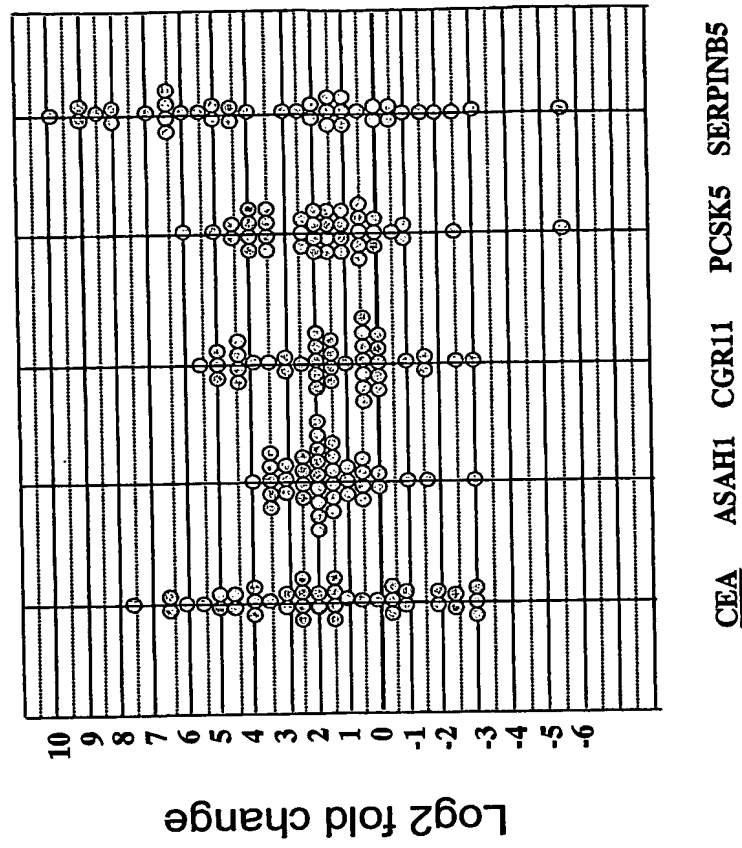


Fig. 10a adlcan

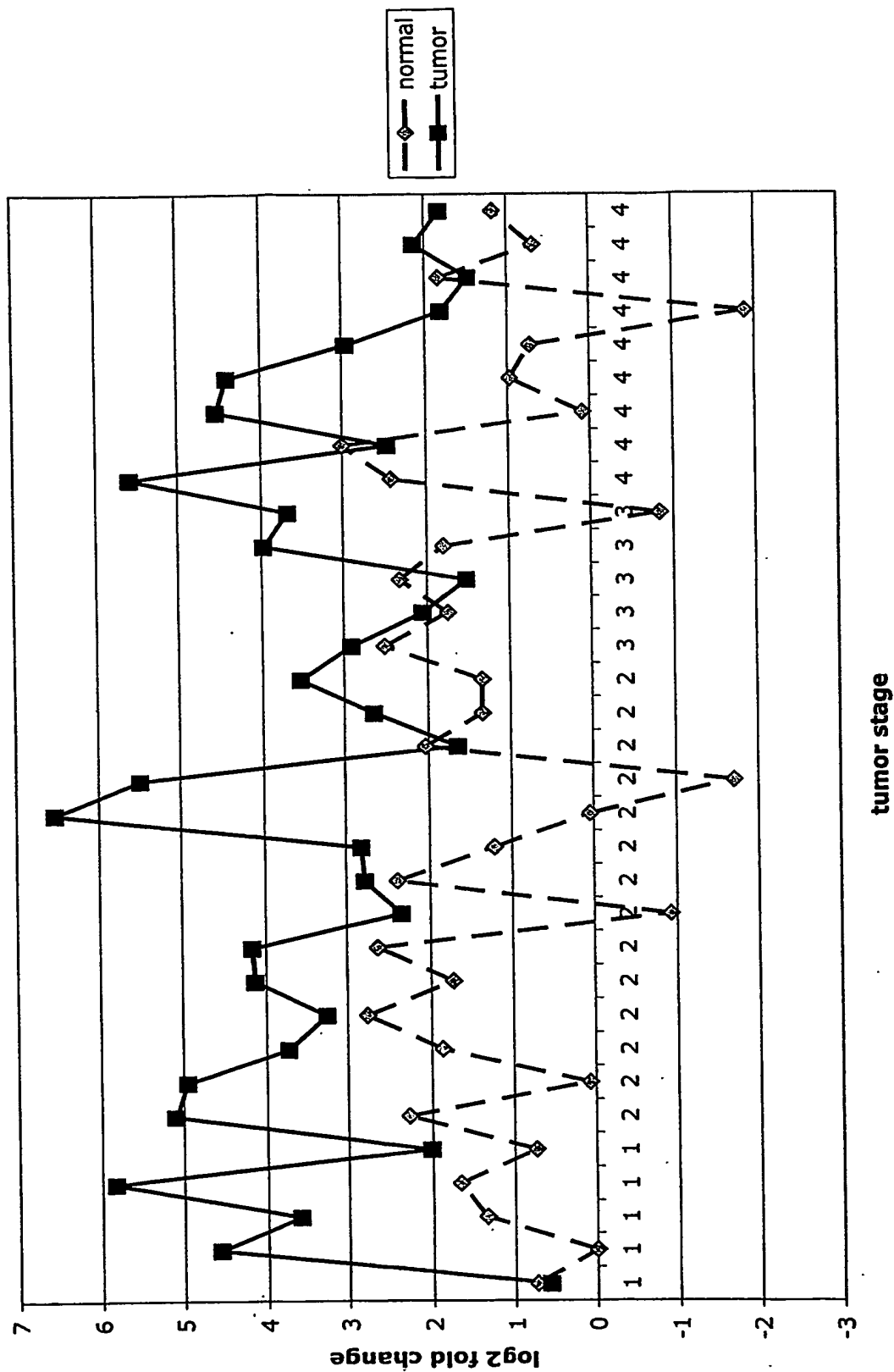




Fig. 10b ASPN

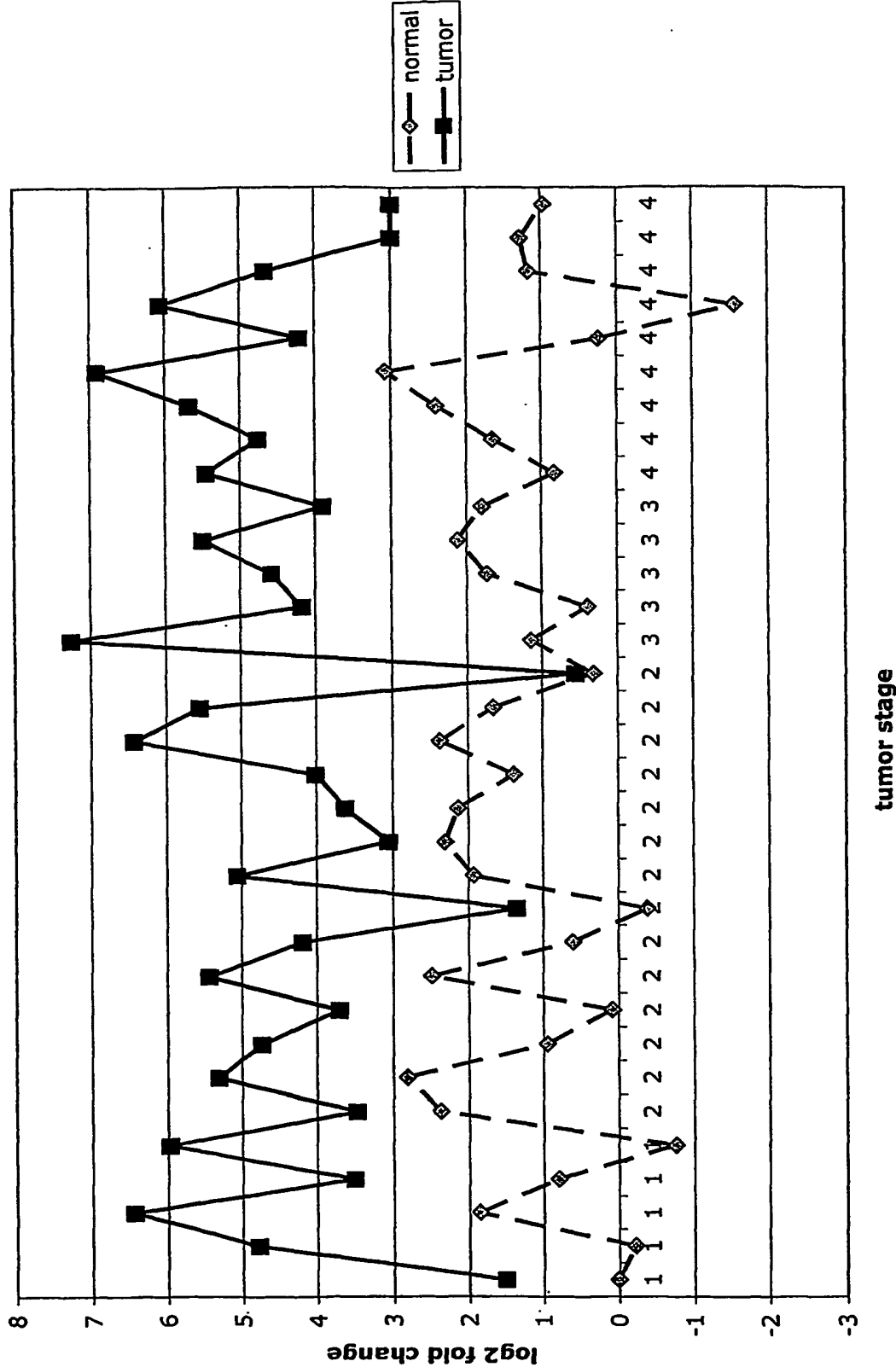


Fig. 10c CSPG2

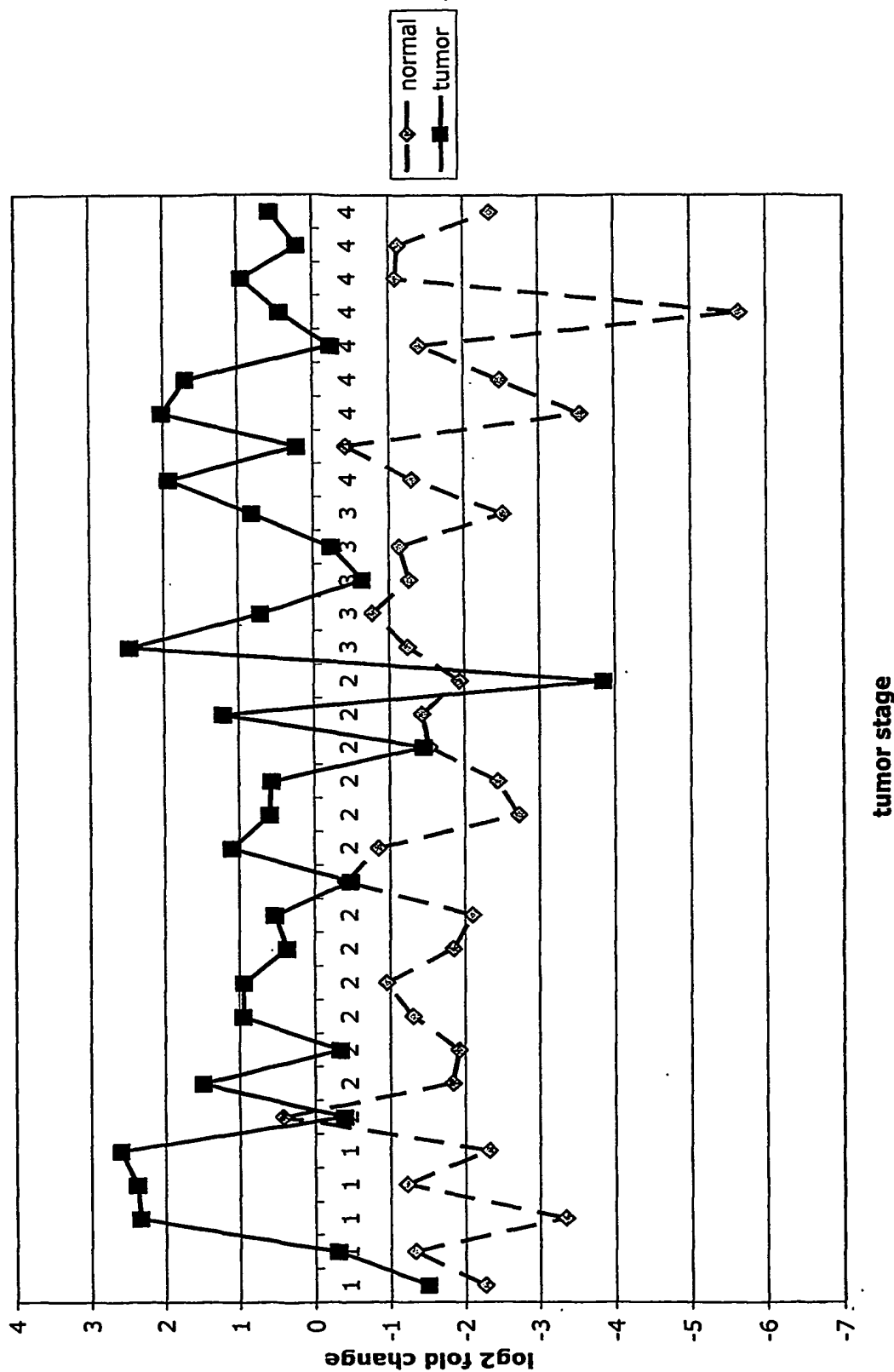


Fig. 10d CST1,2,4

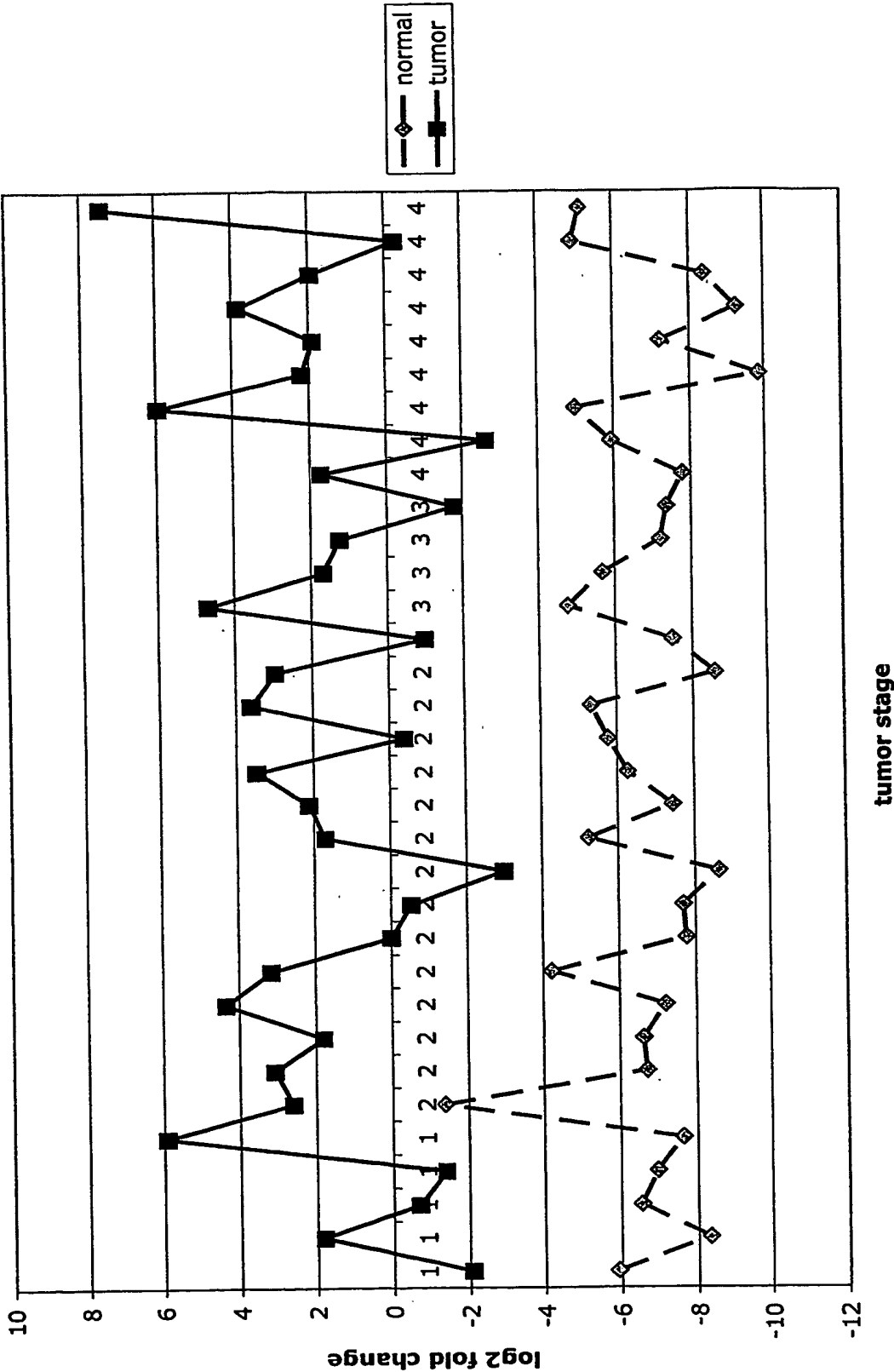


Fig. 10e EFEMP2

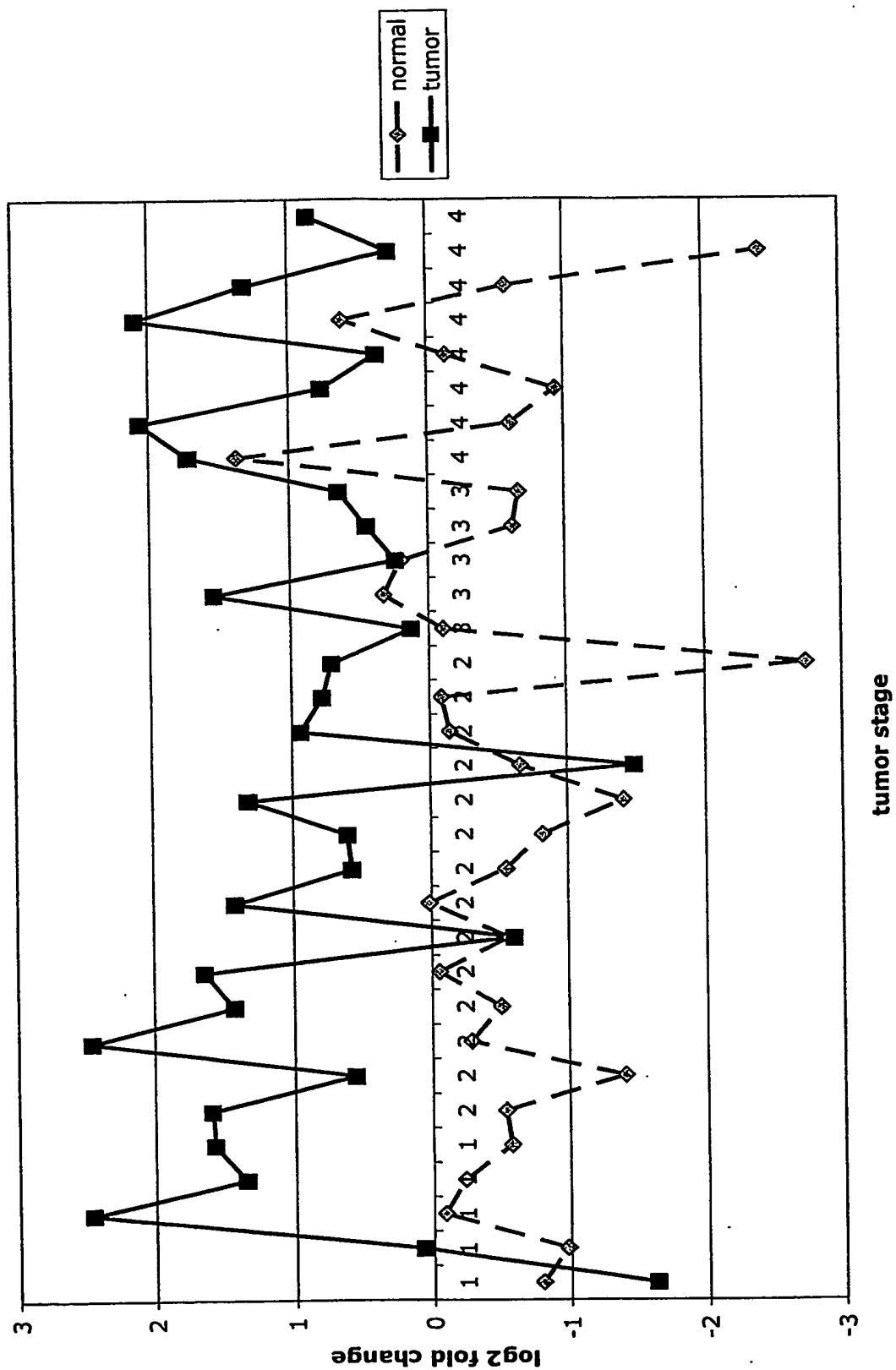


Fig. 10f GGH

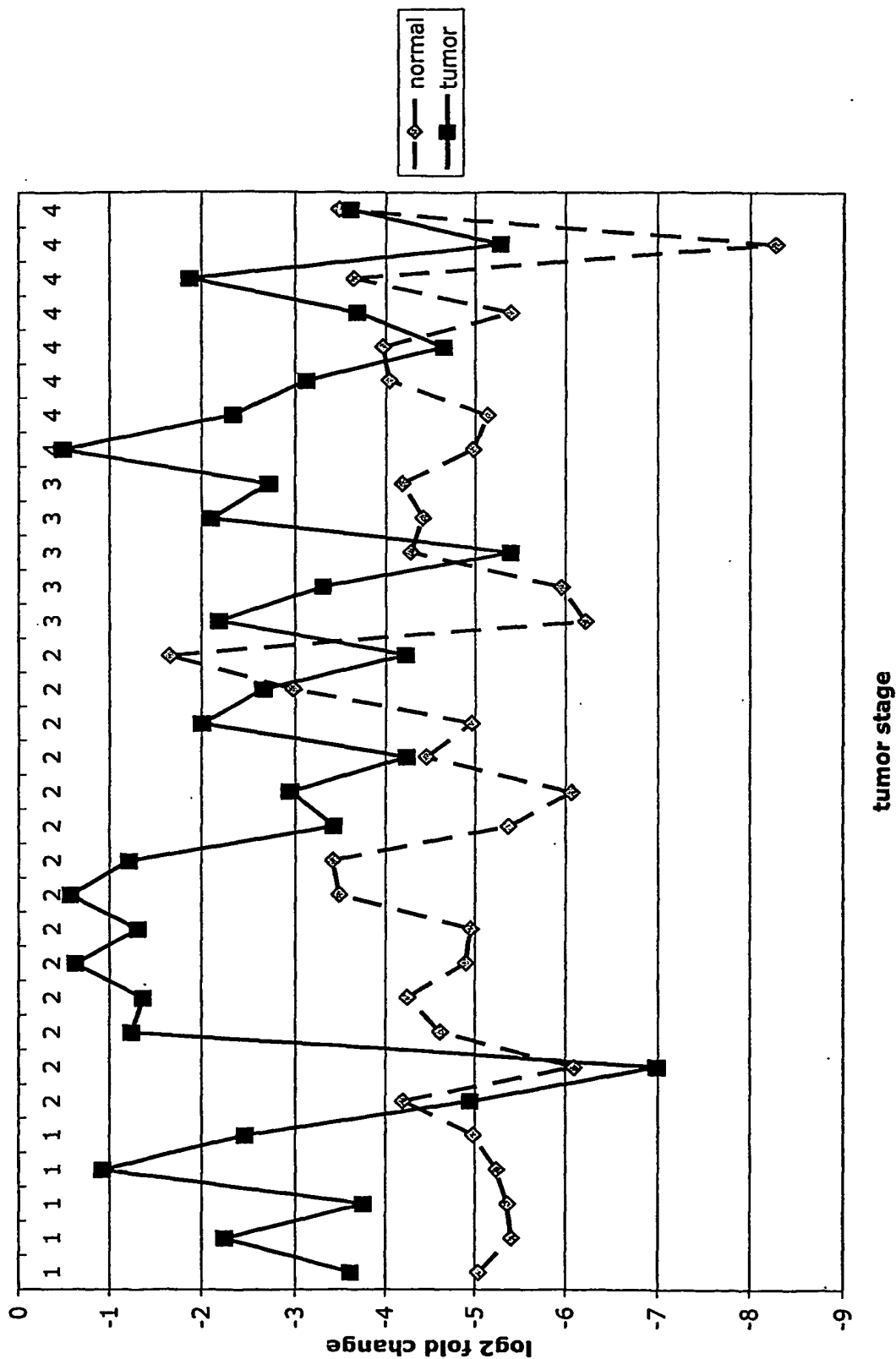


Fig. 10g INHBA

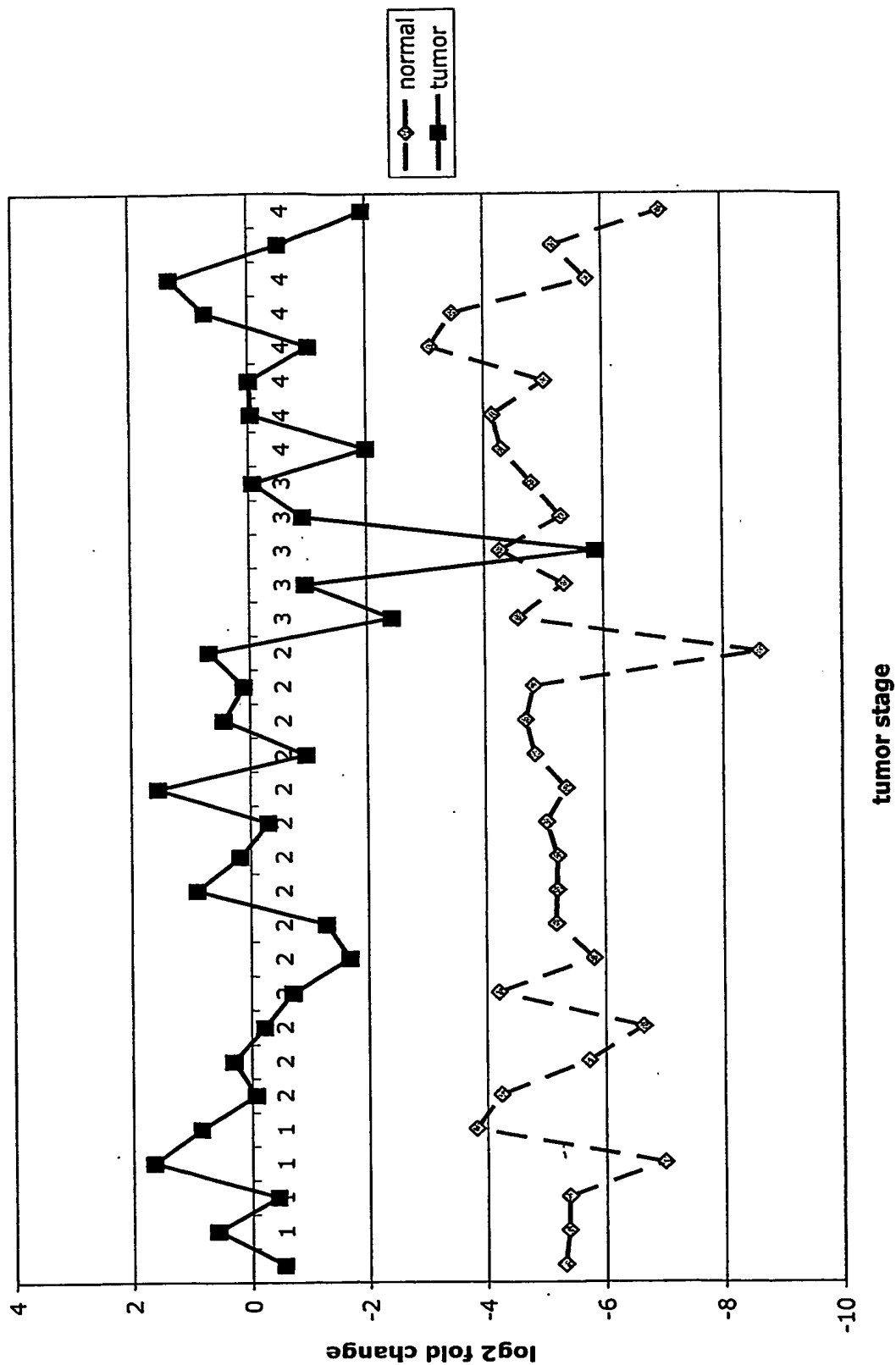


Fig. 10h IGFBP7

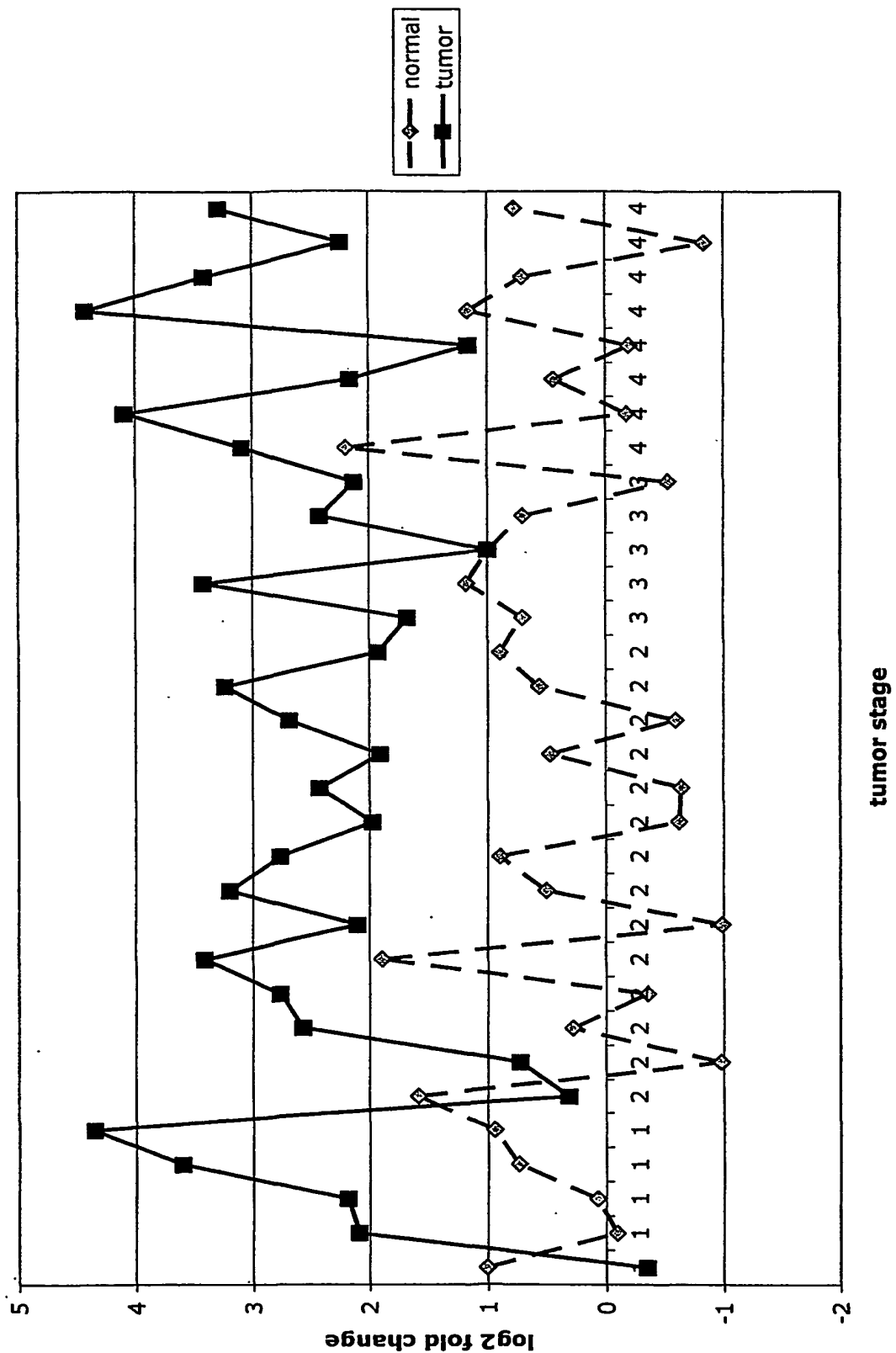


Fig. 10i KLK10

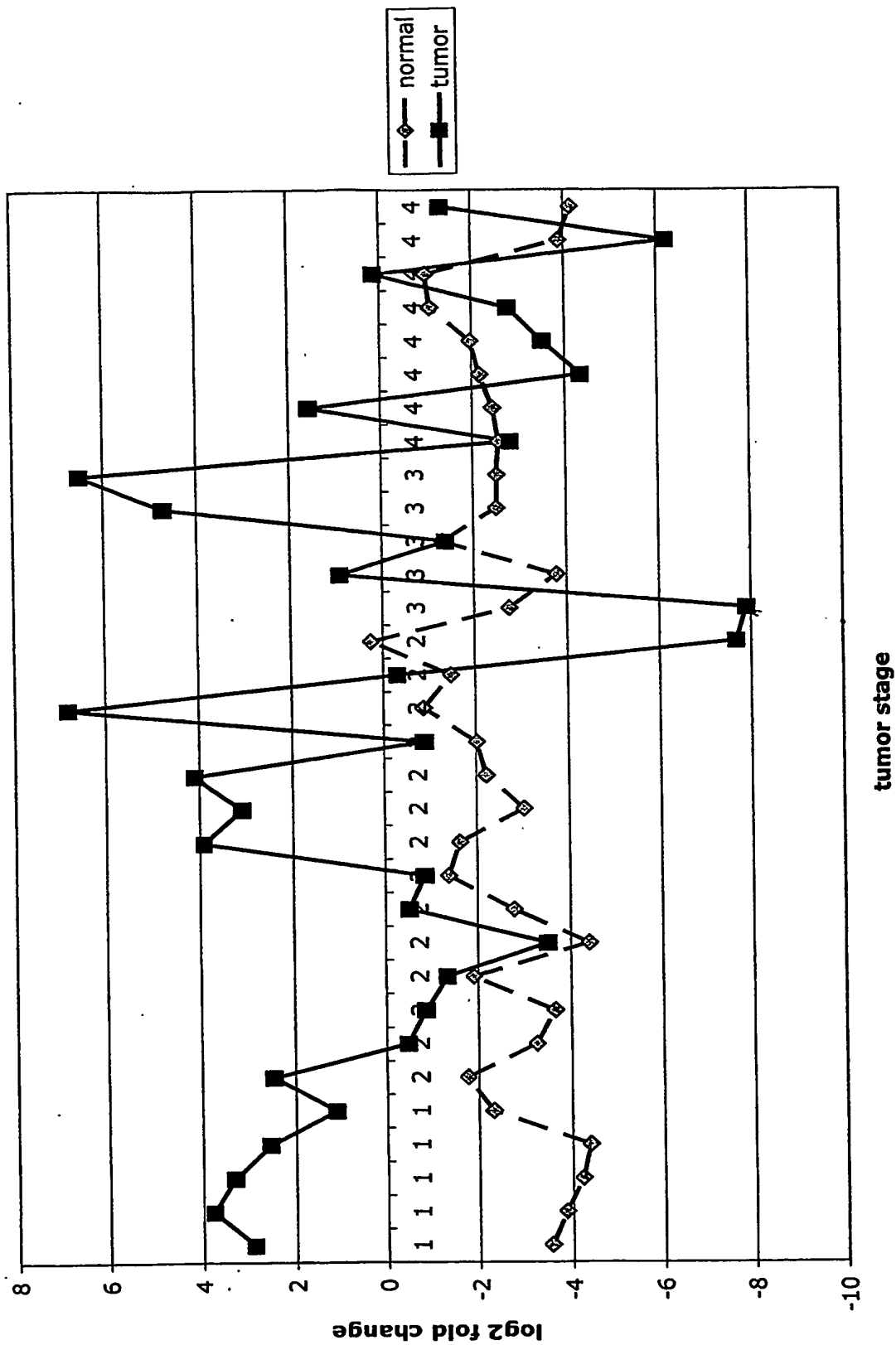




Fig. 10j LEPRE1

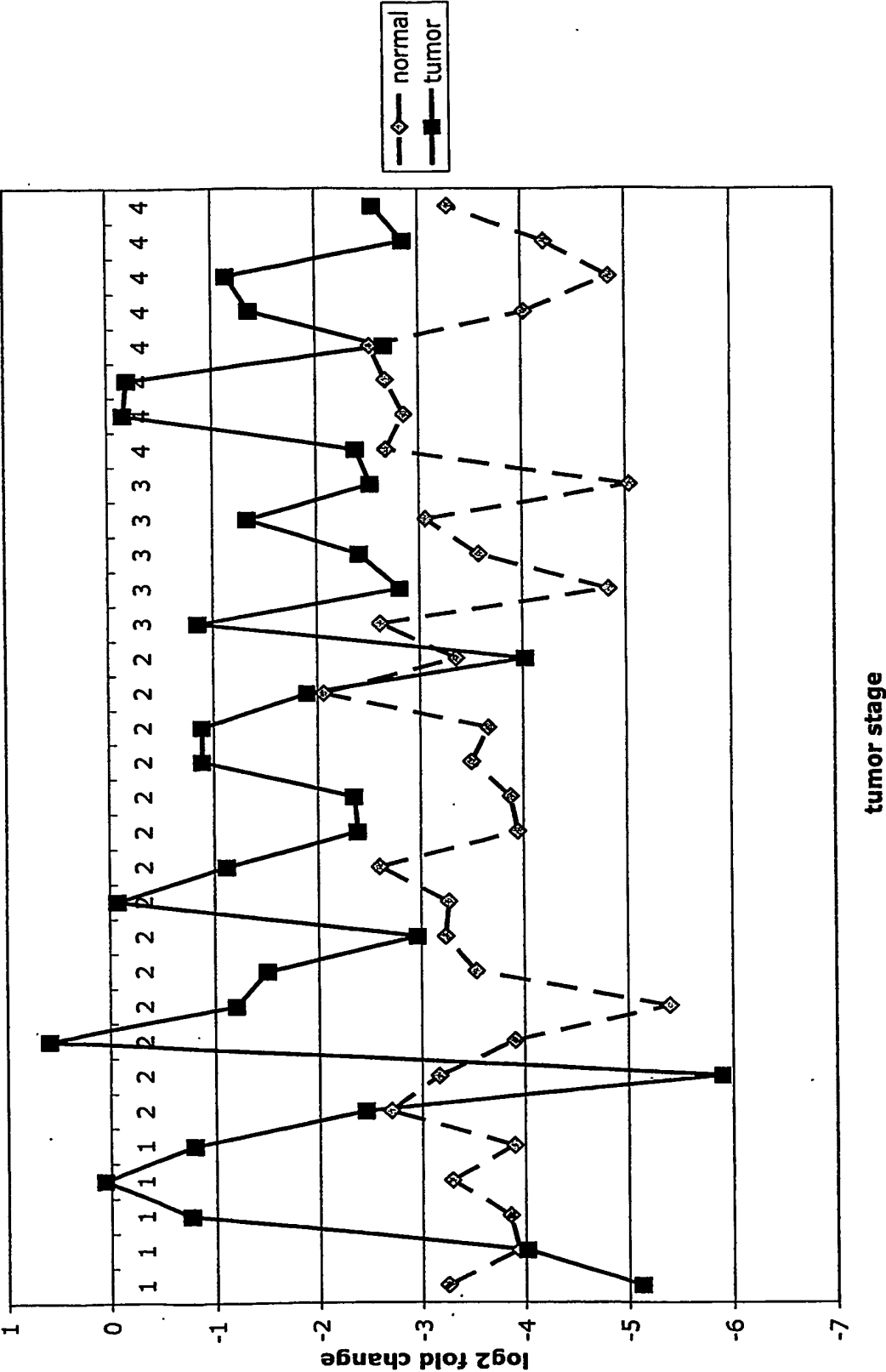


Fig. 10k LUM

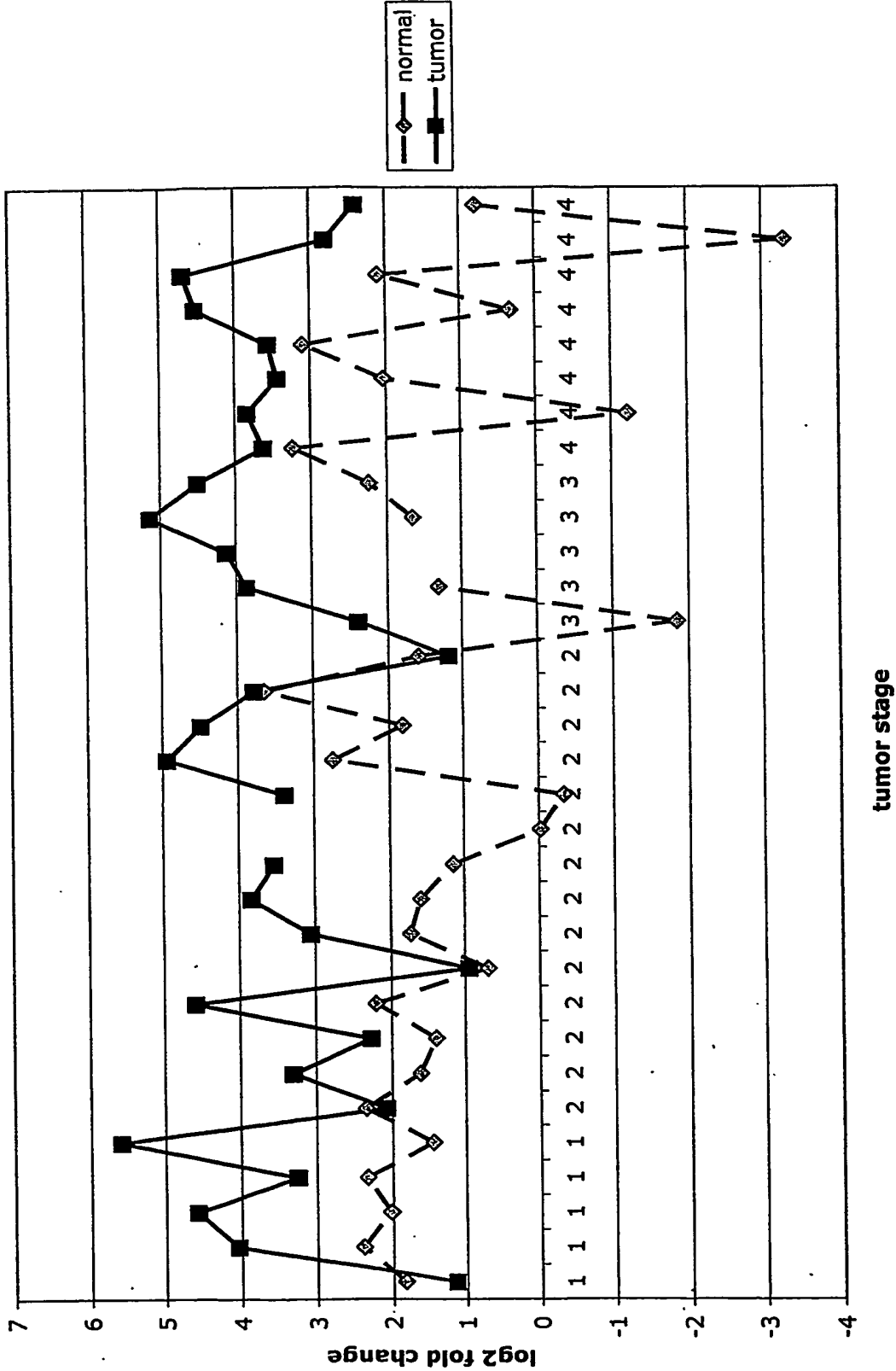


Fig. 10I LOXL2

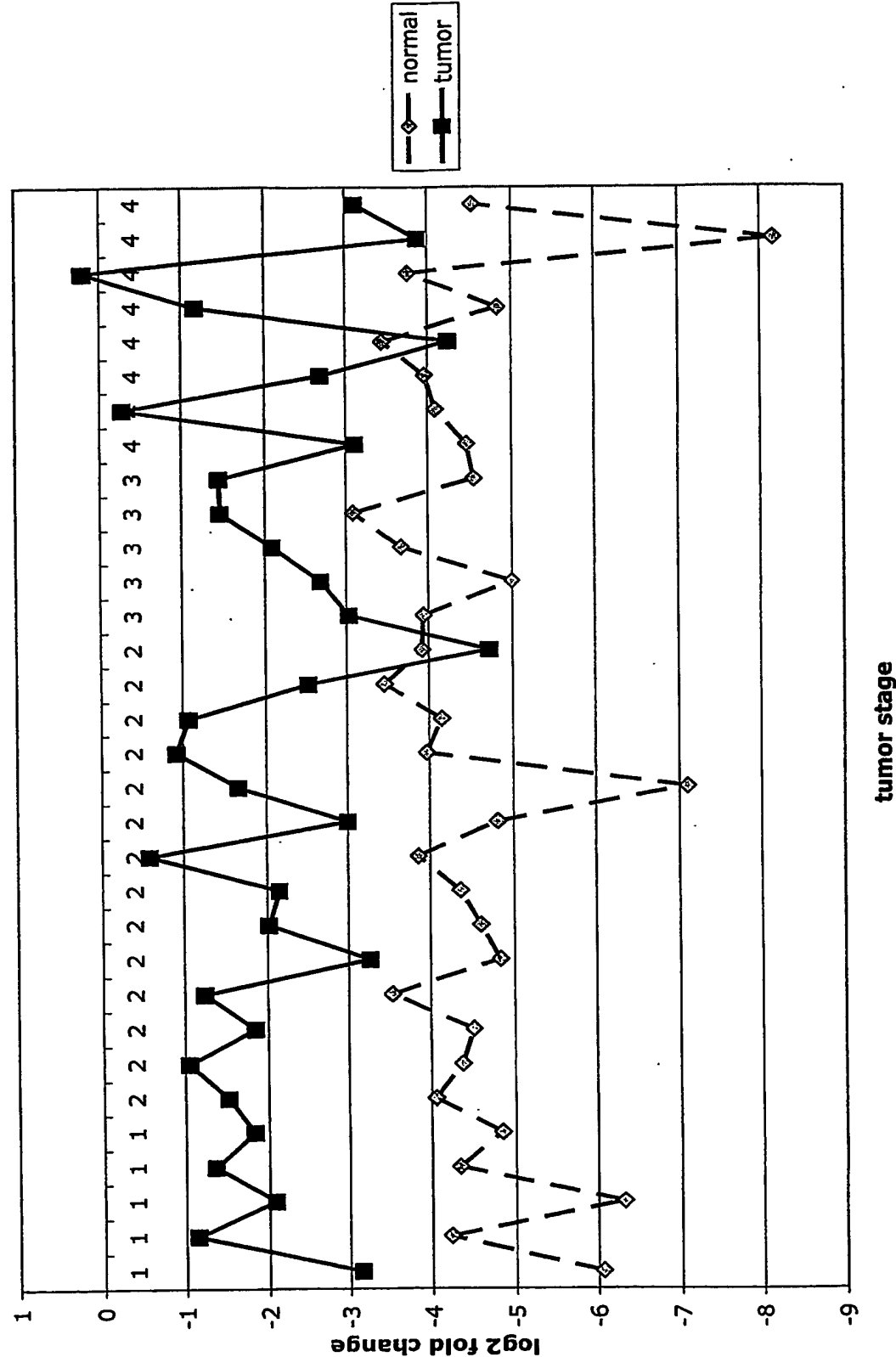


Fig. 10m MMP12

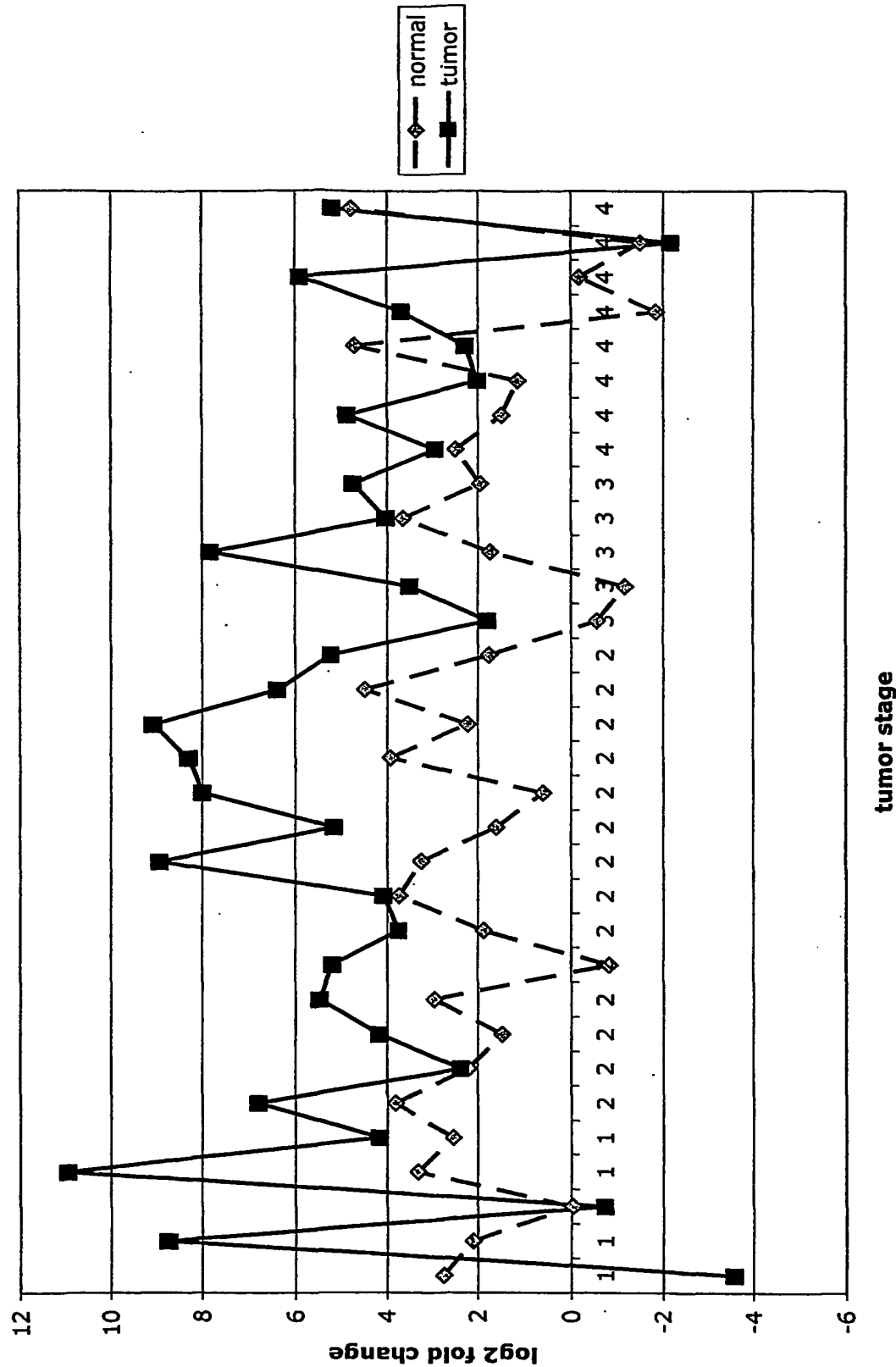


Fig.10n TIMP1

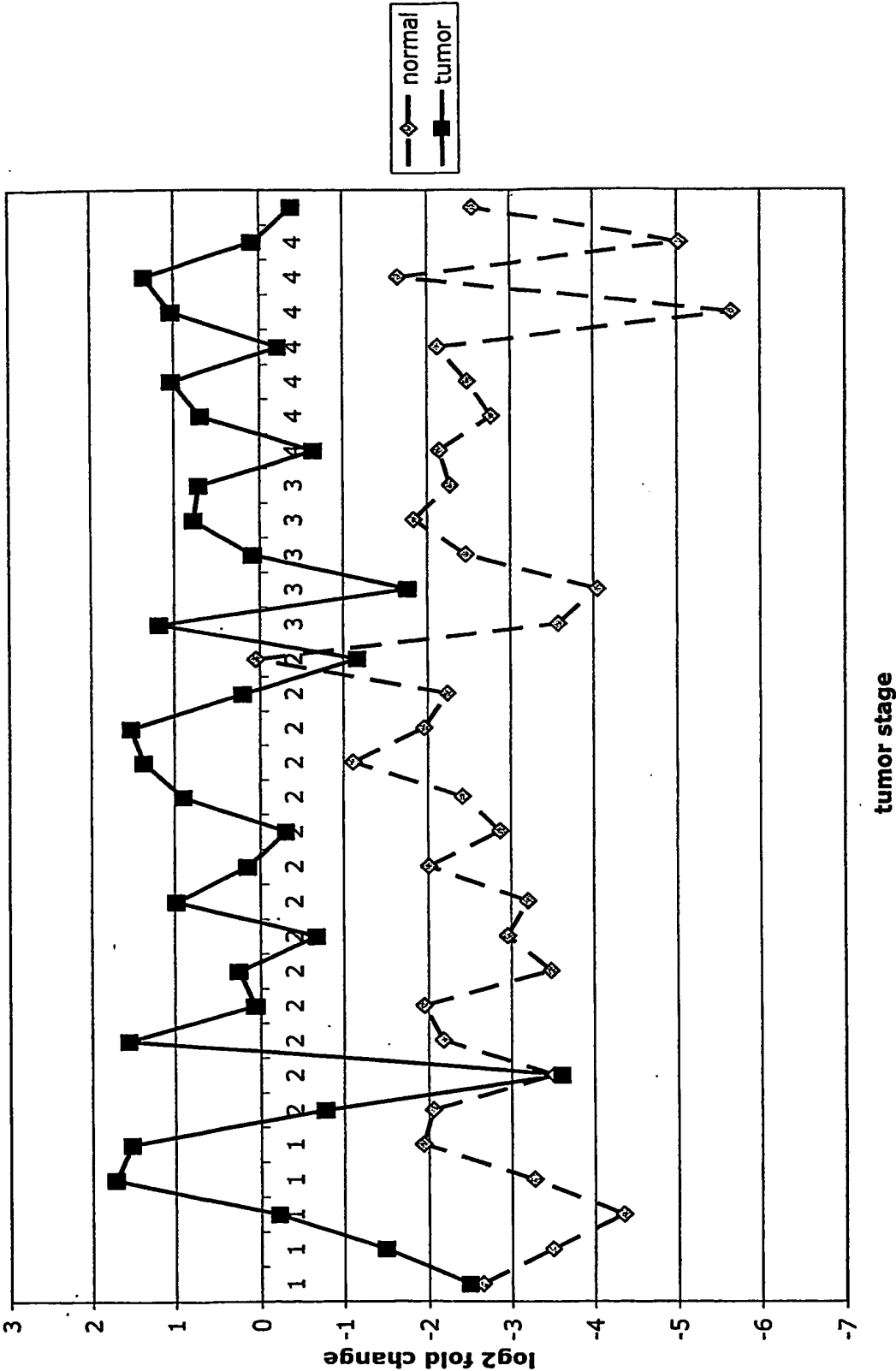


Fig. 10o ASAH1

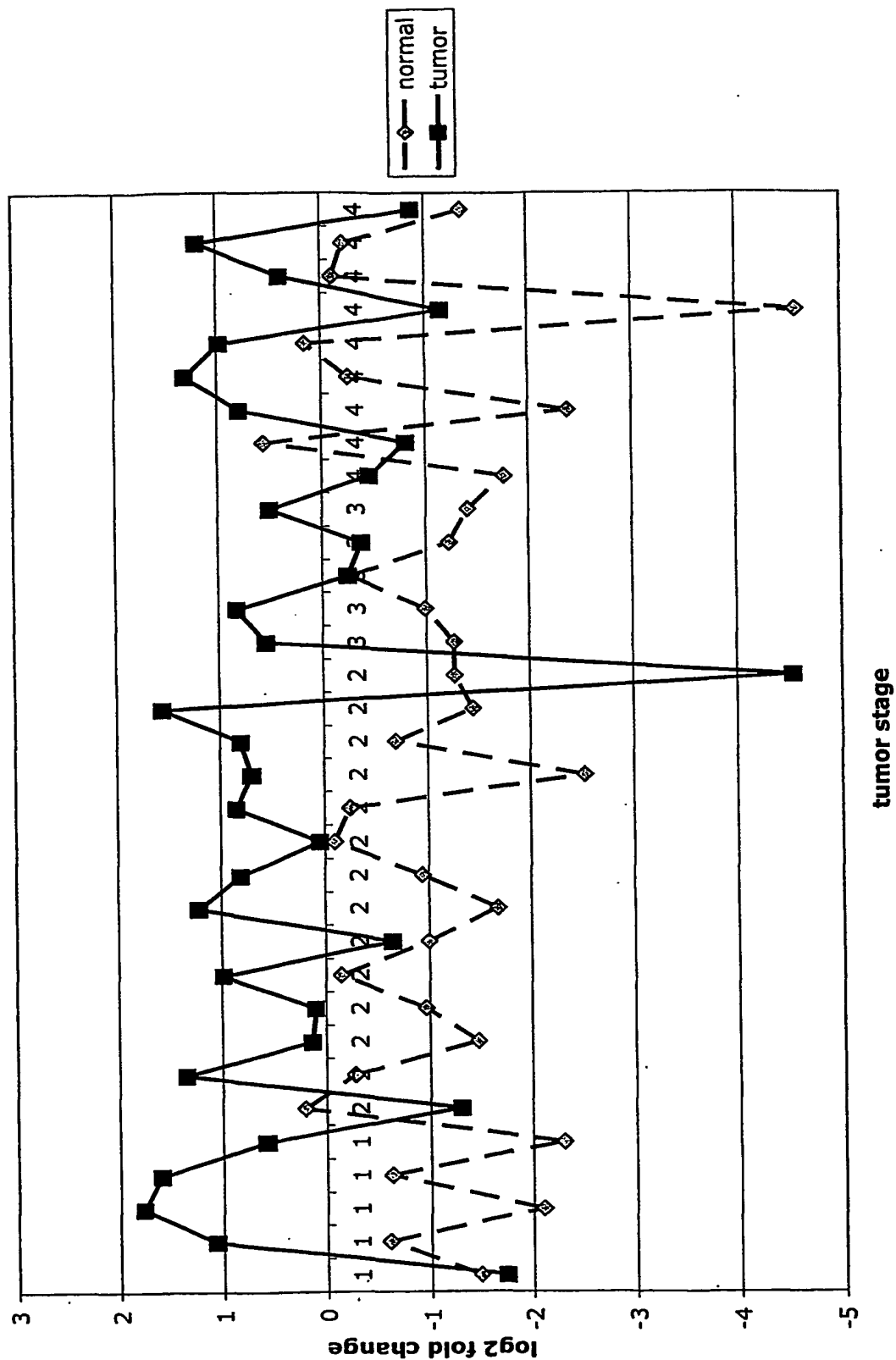


Fig. 10p SPP1

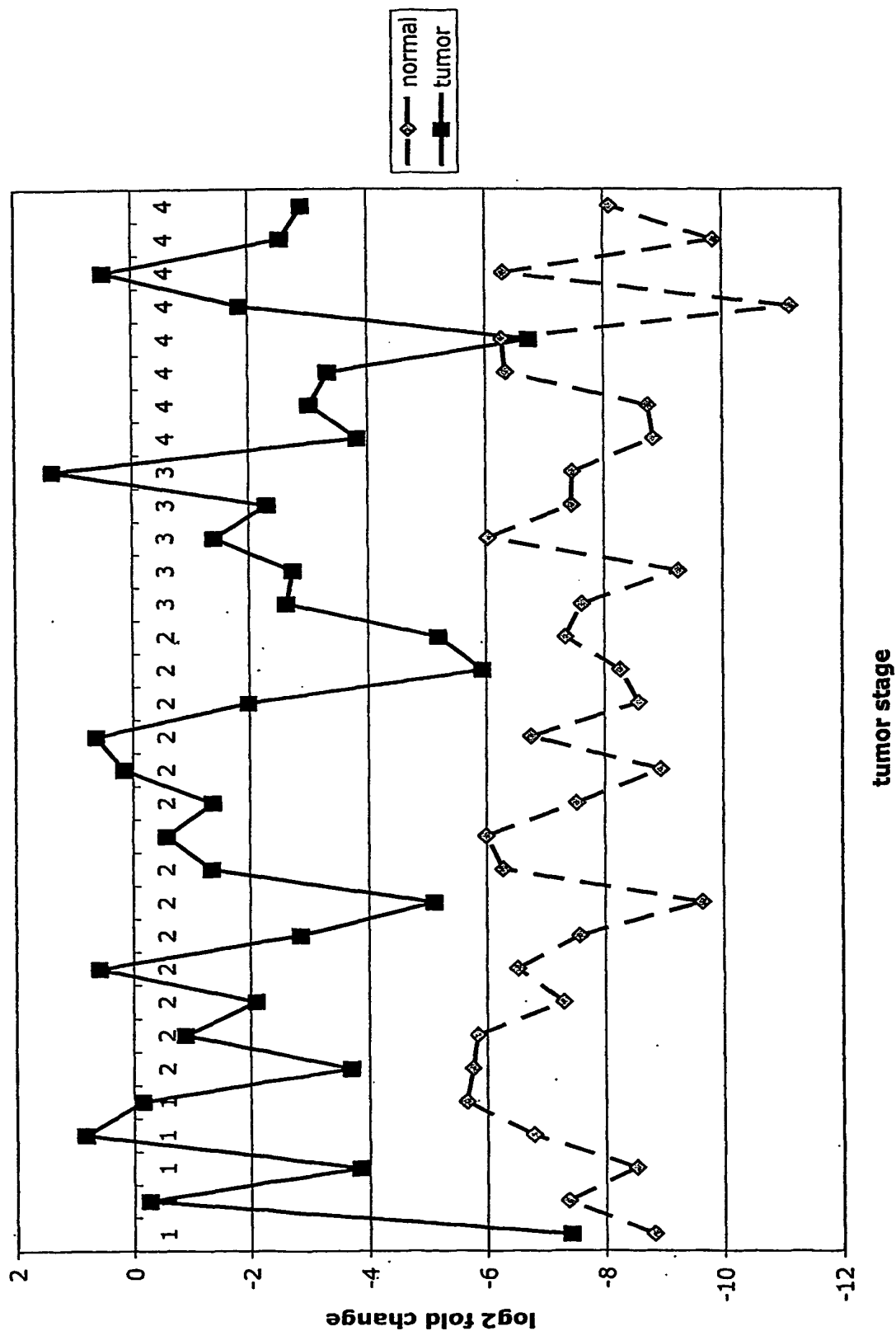


Fig. 10q SFRP2

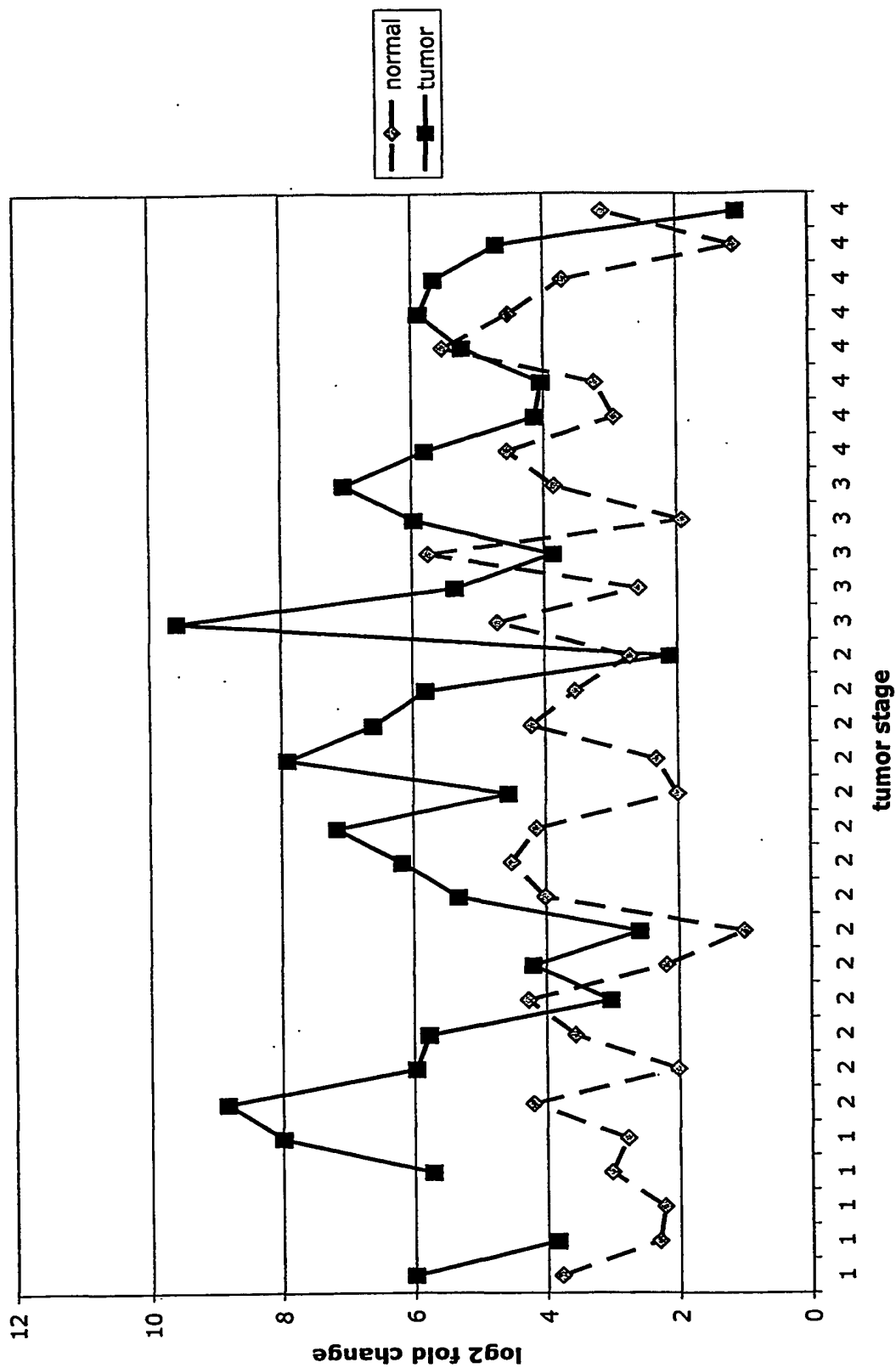




Fig. 10r SFRP4

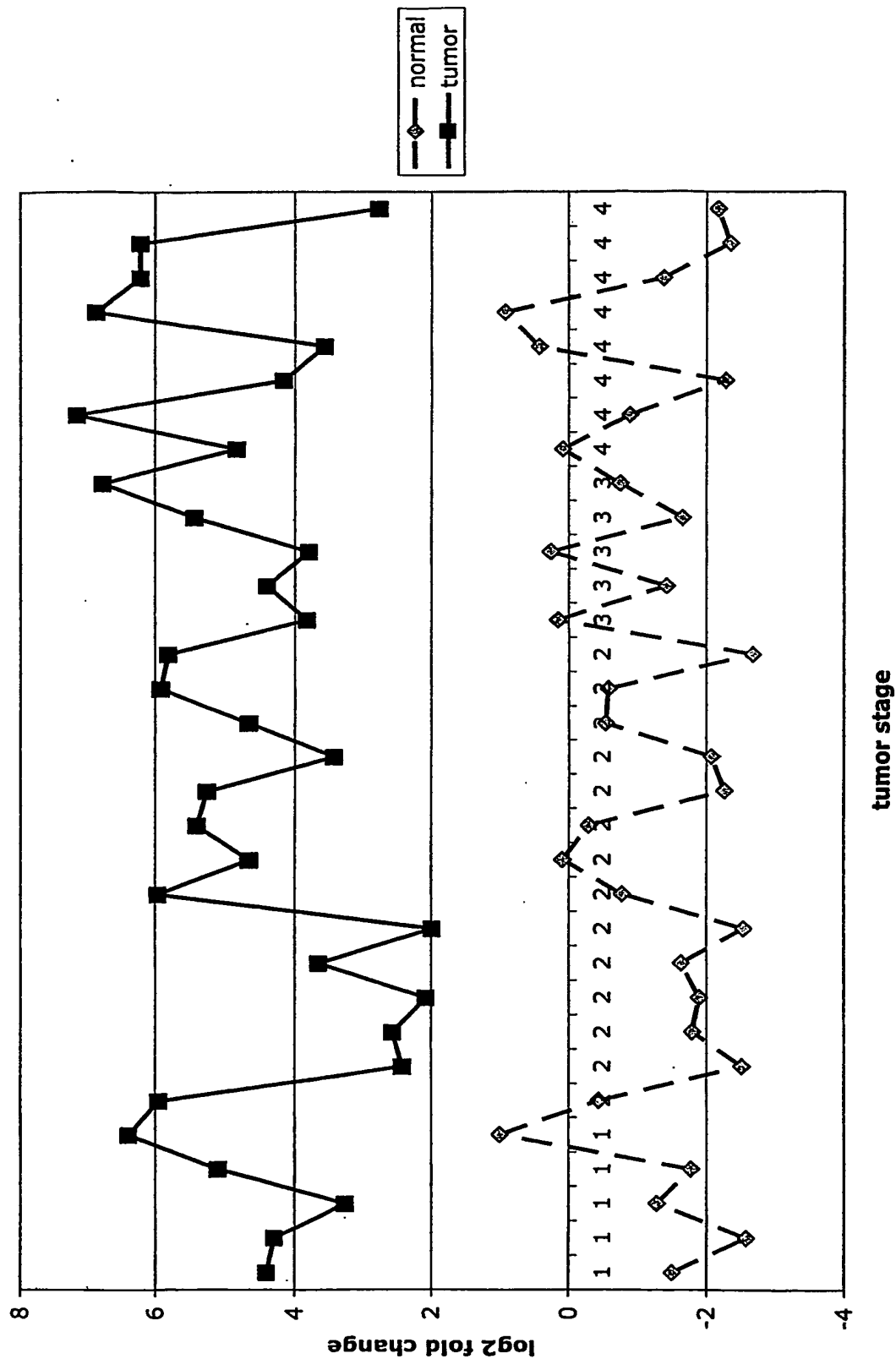


Fig. 10s SPARC

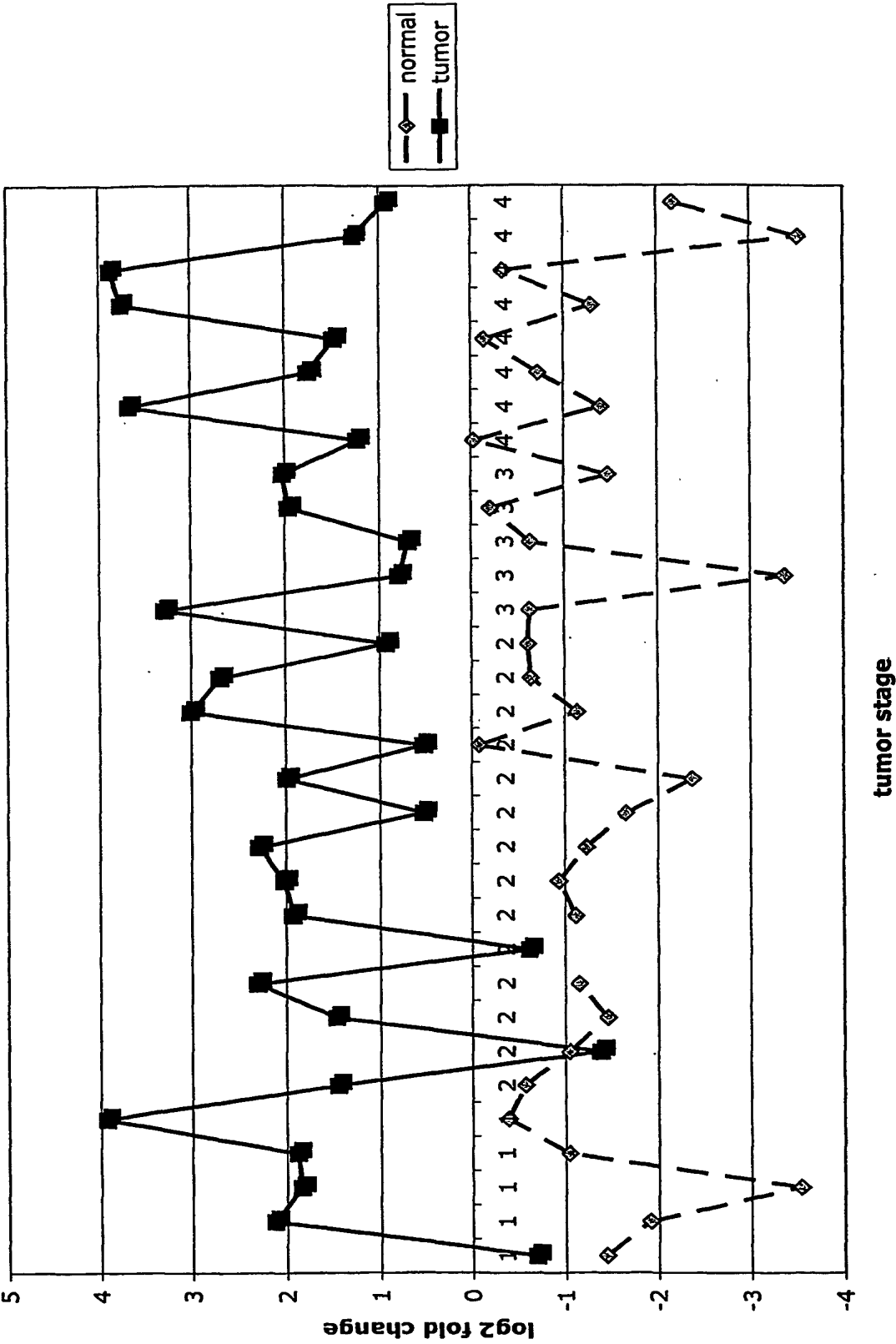


Fig. 10t PRSS11

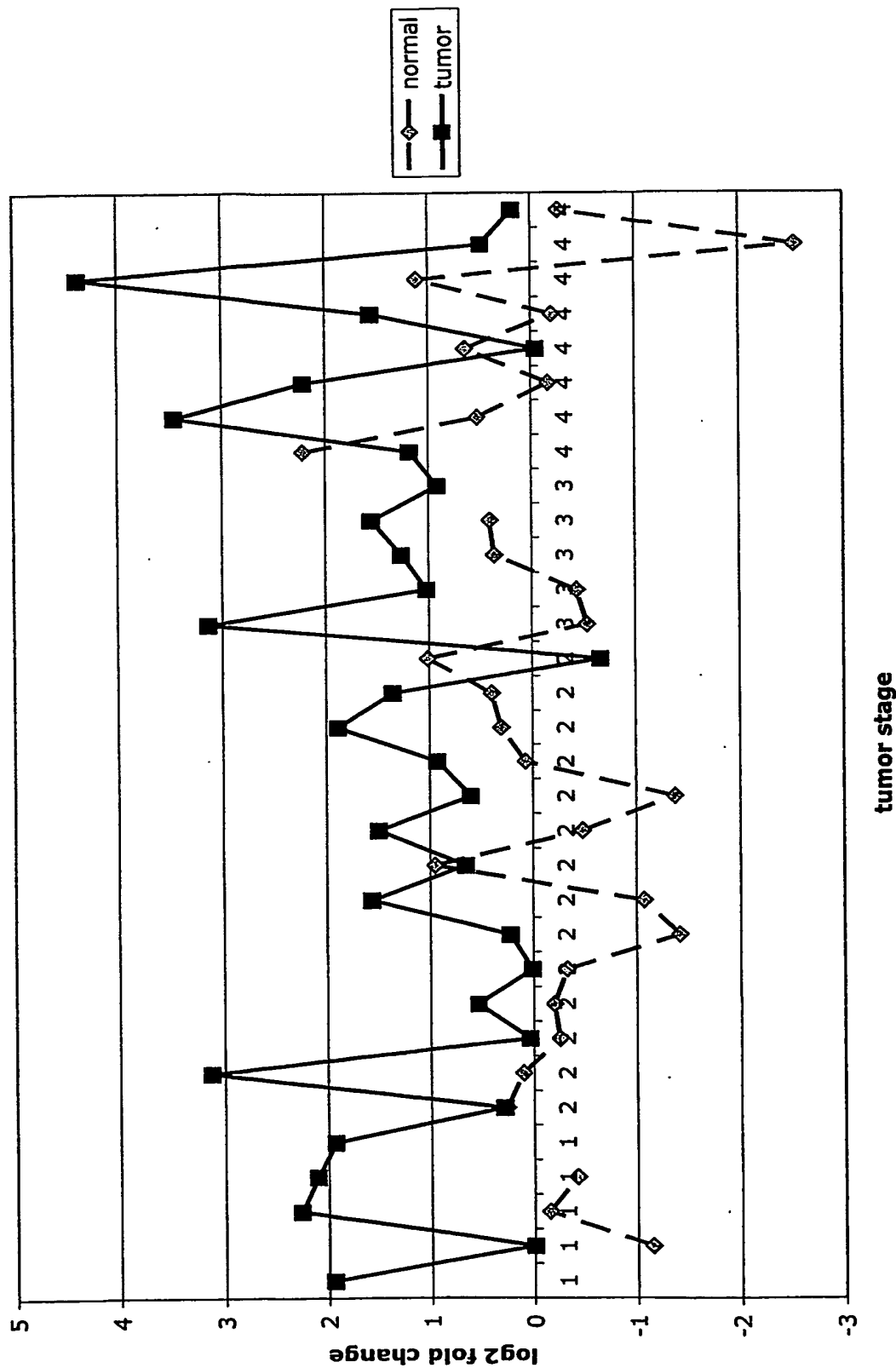
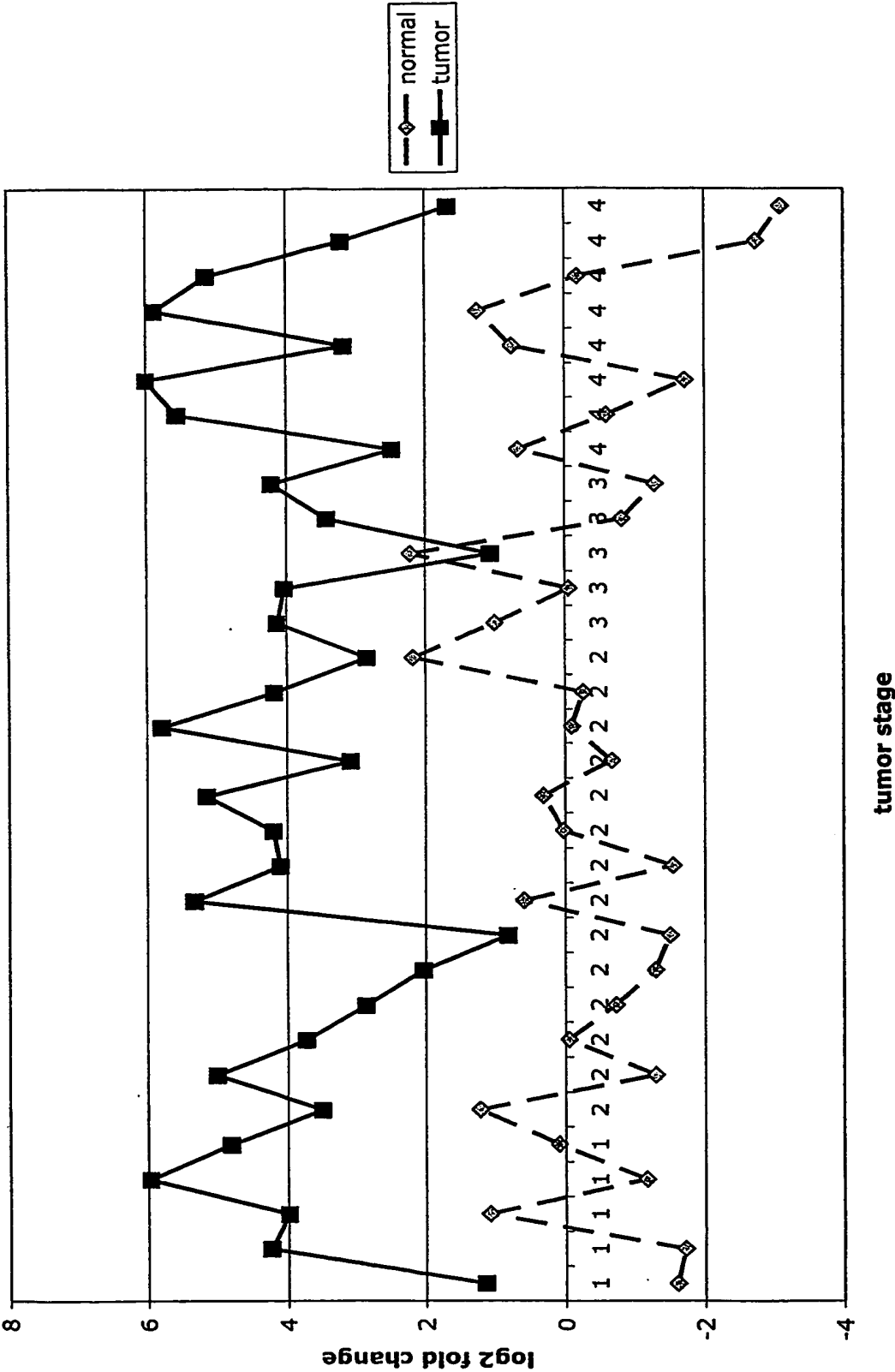


Fig. 10u THBS2



**Fig. 10v TG**

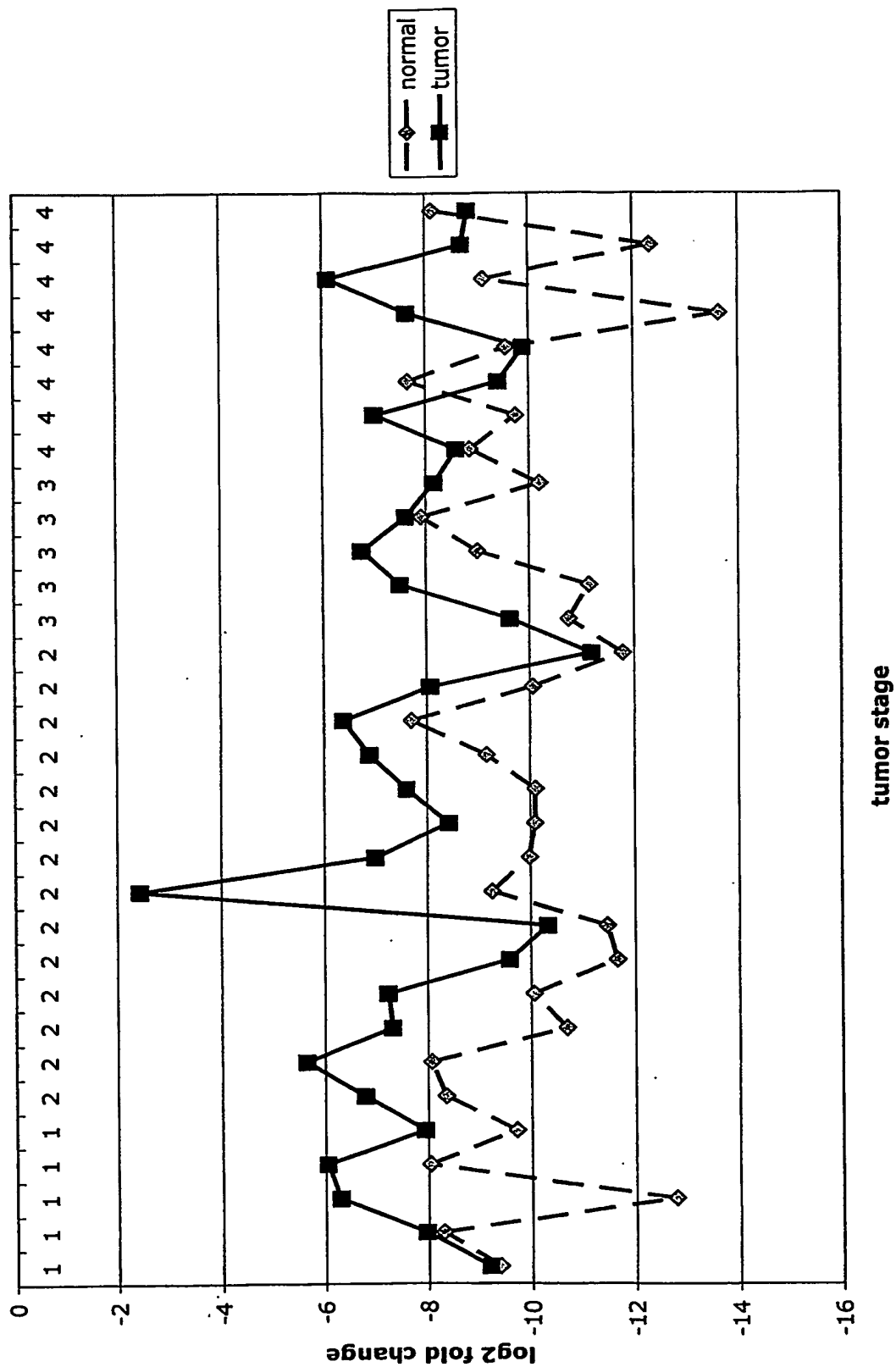


Fig. 10w TGFB1

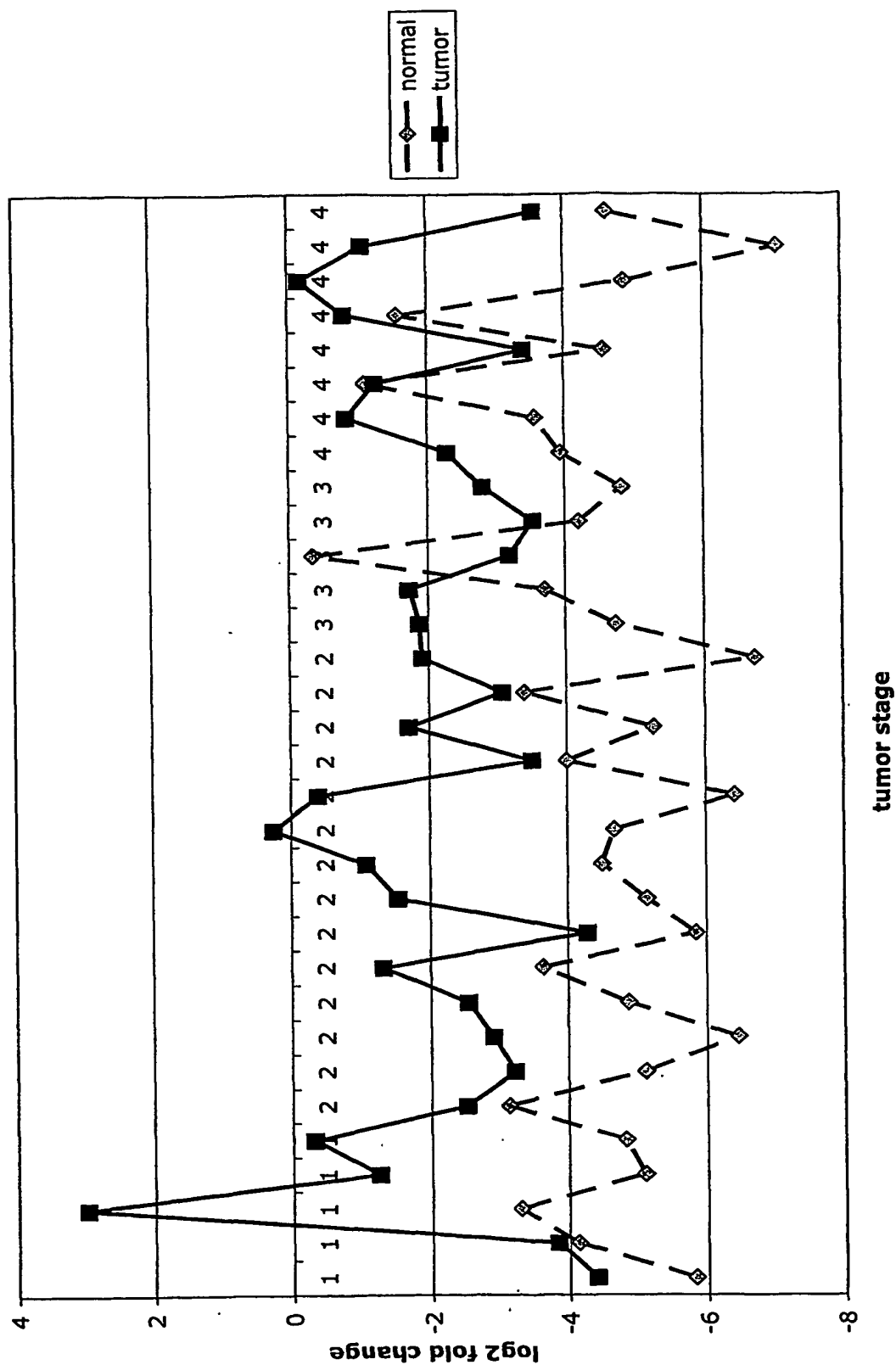


Fig. 10x CGR11

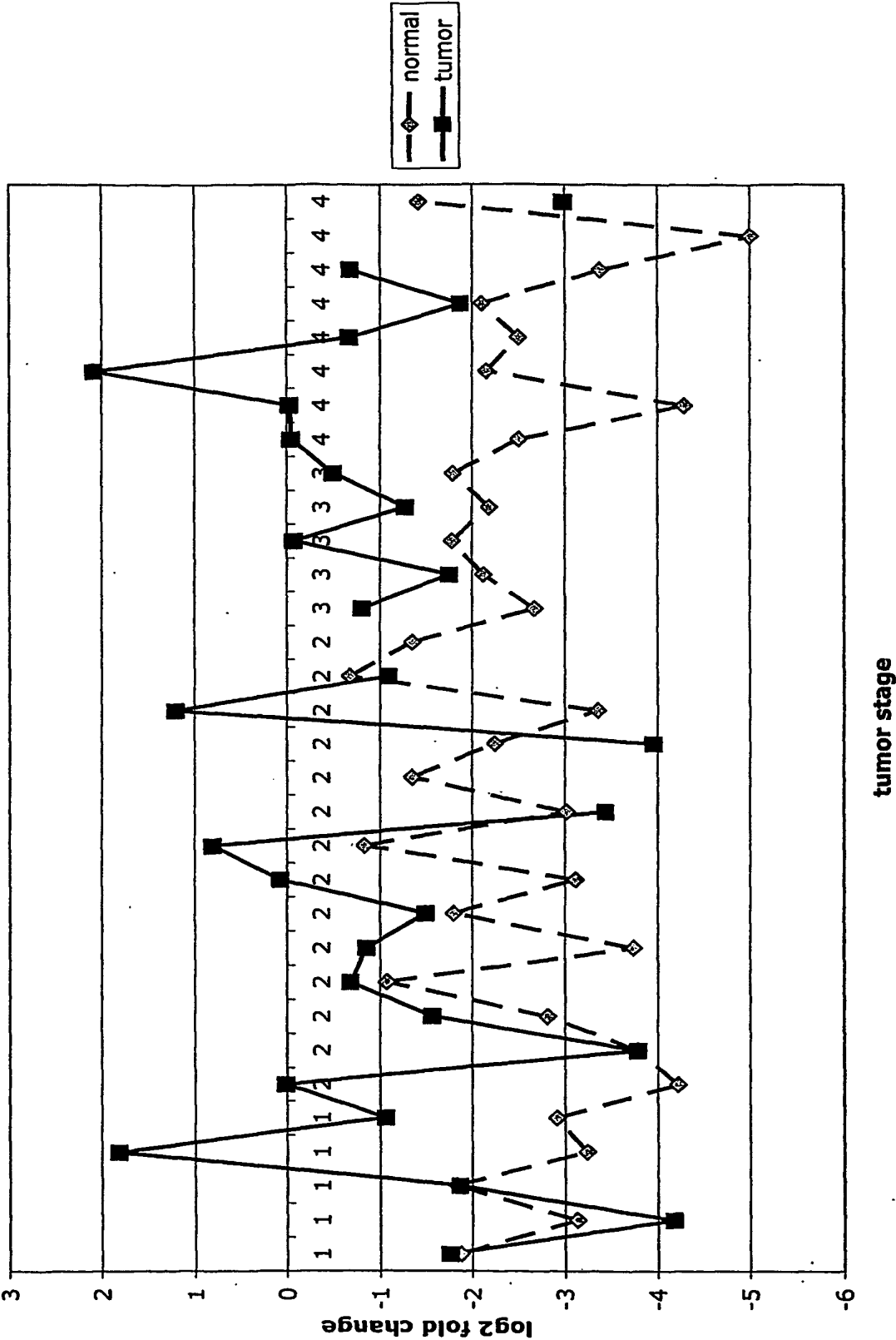


Fig. 10y SERPINH1

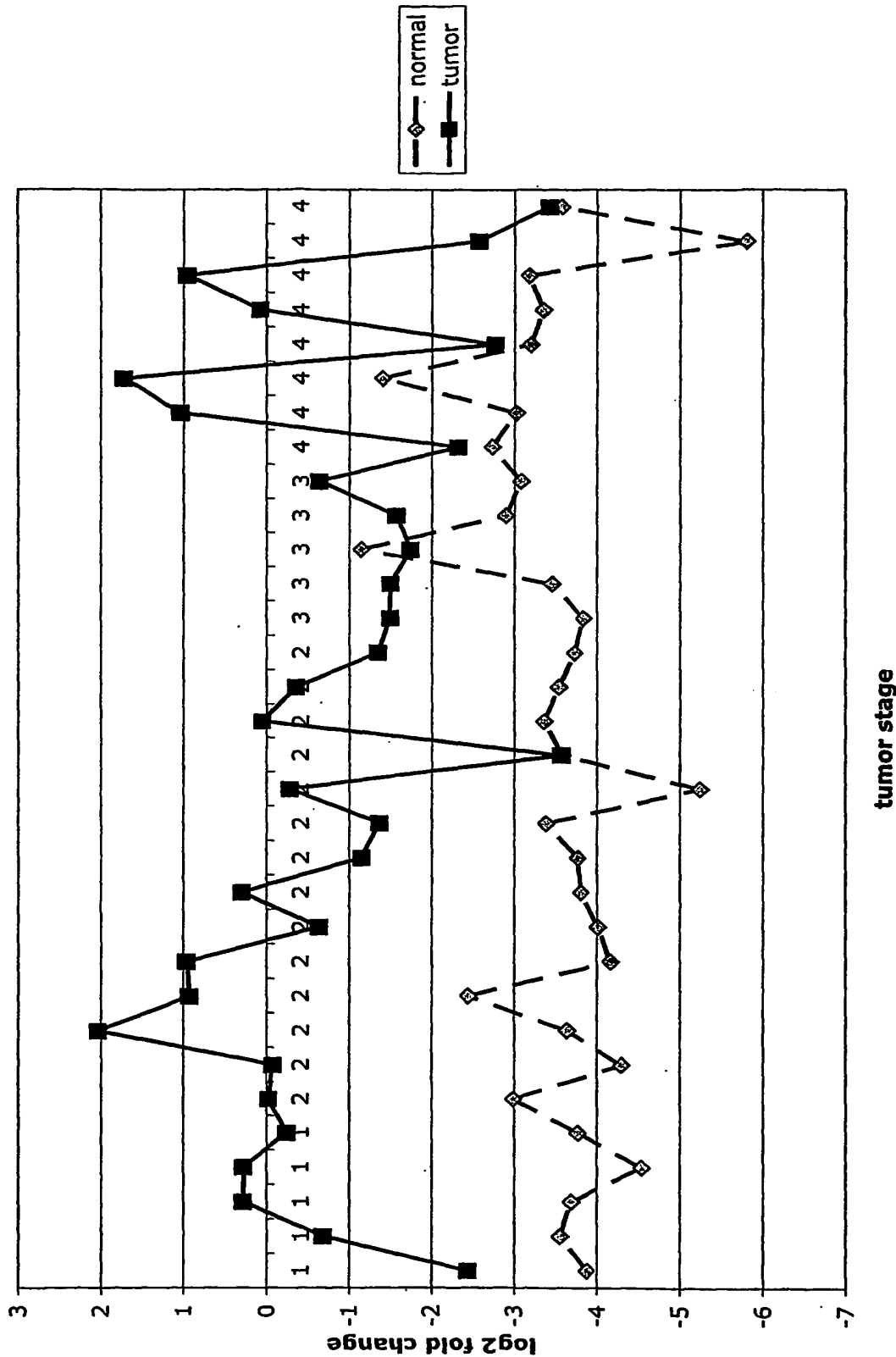




Fig. 10z MMP2

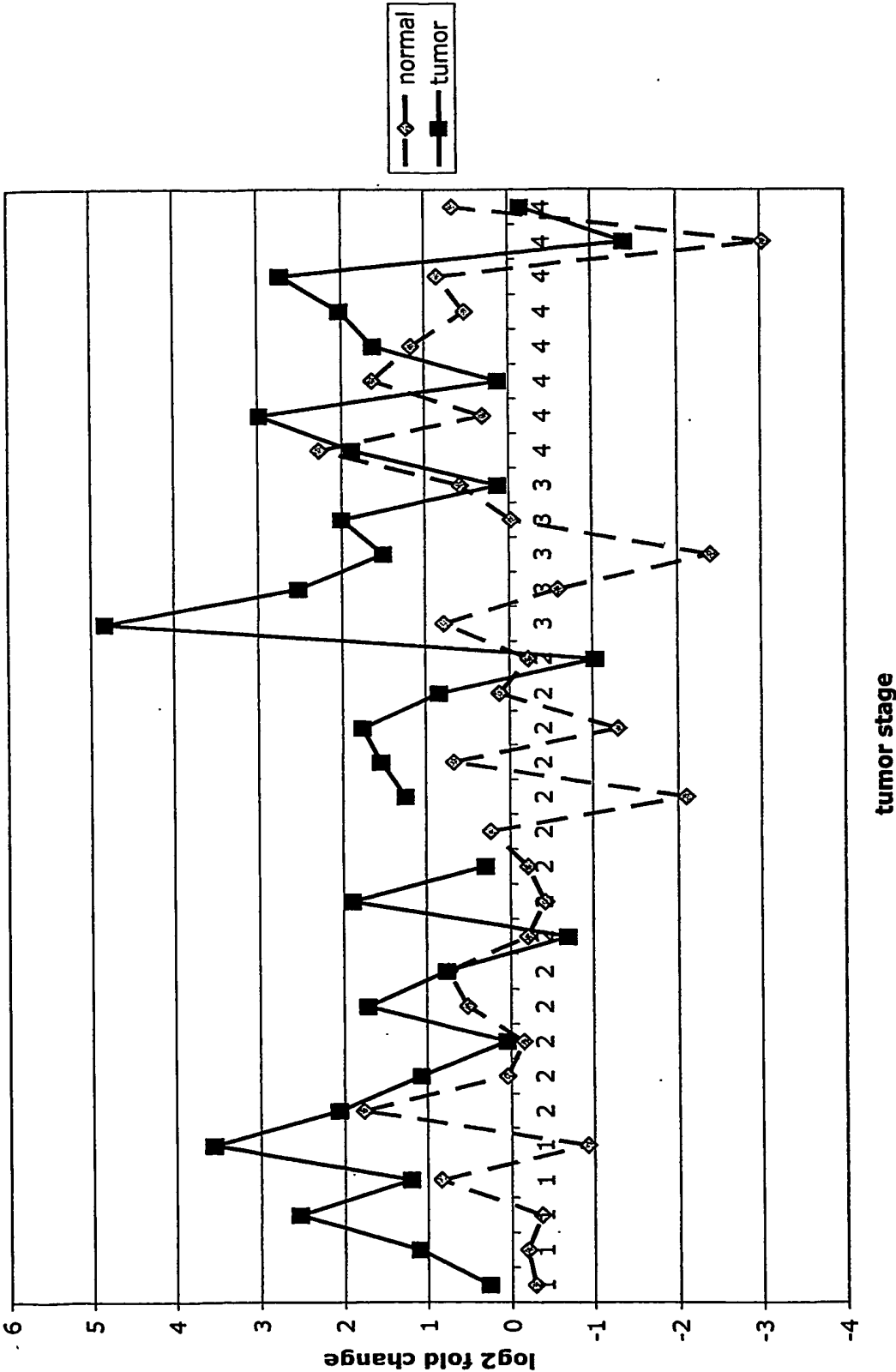


Fig. 10aa PCSK5

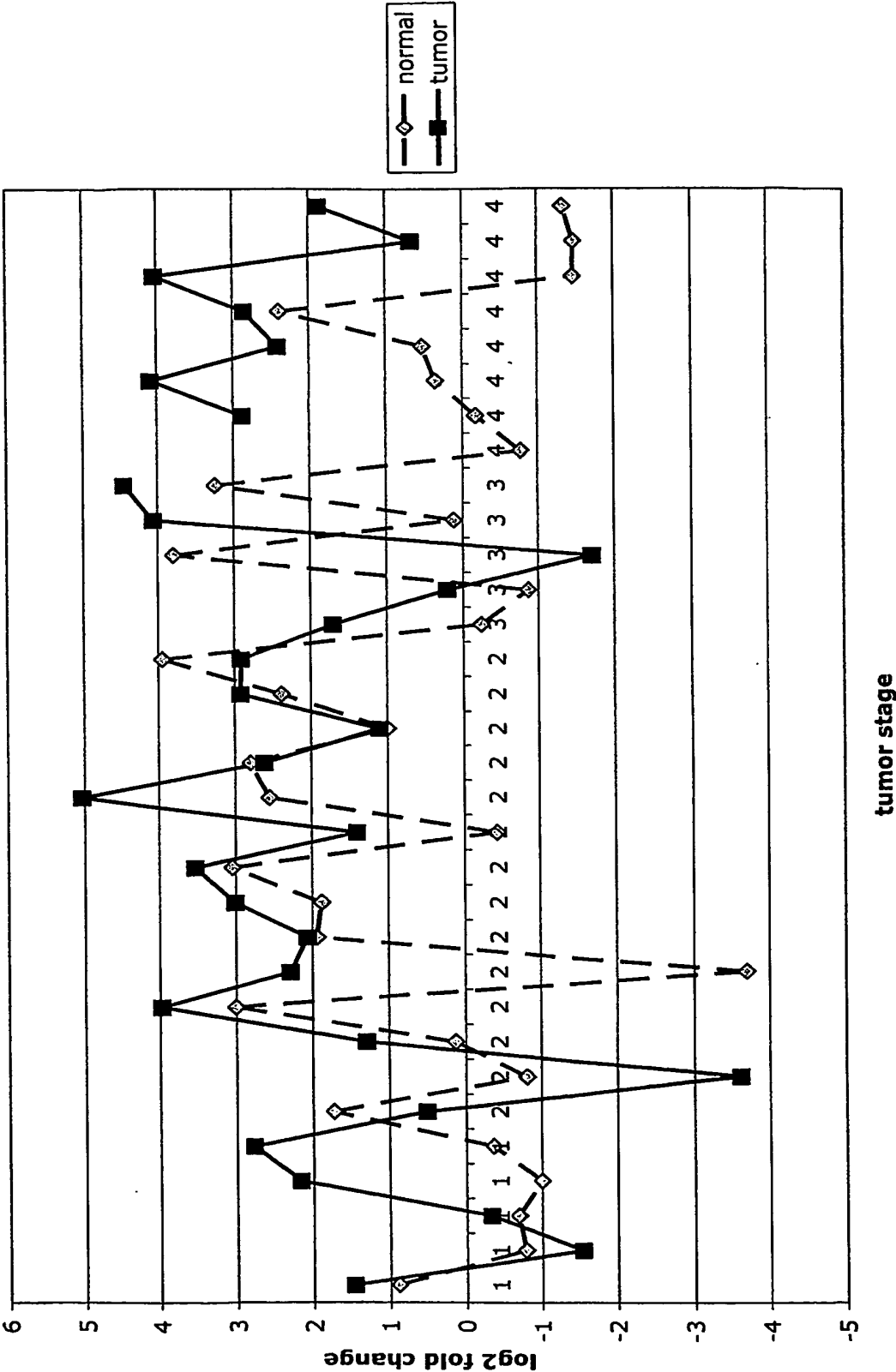
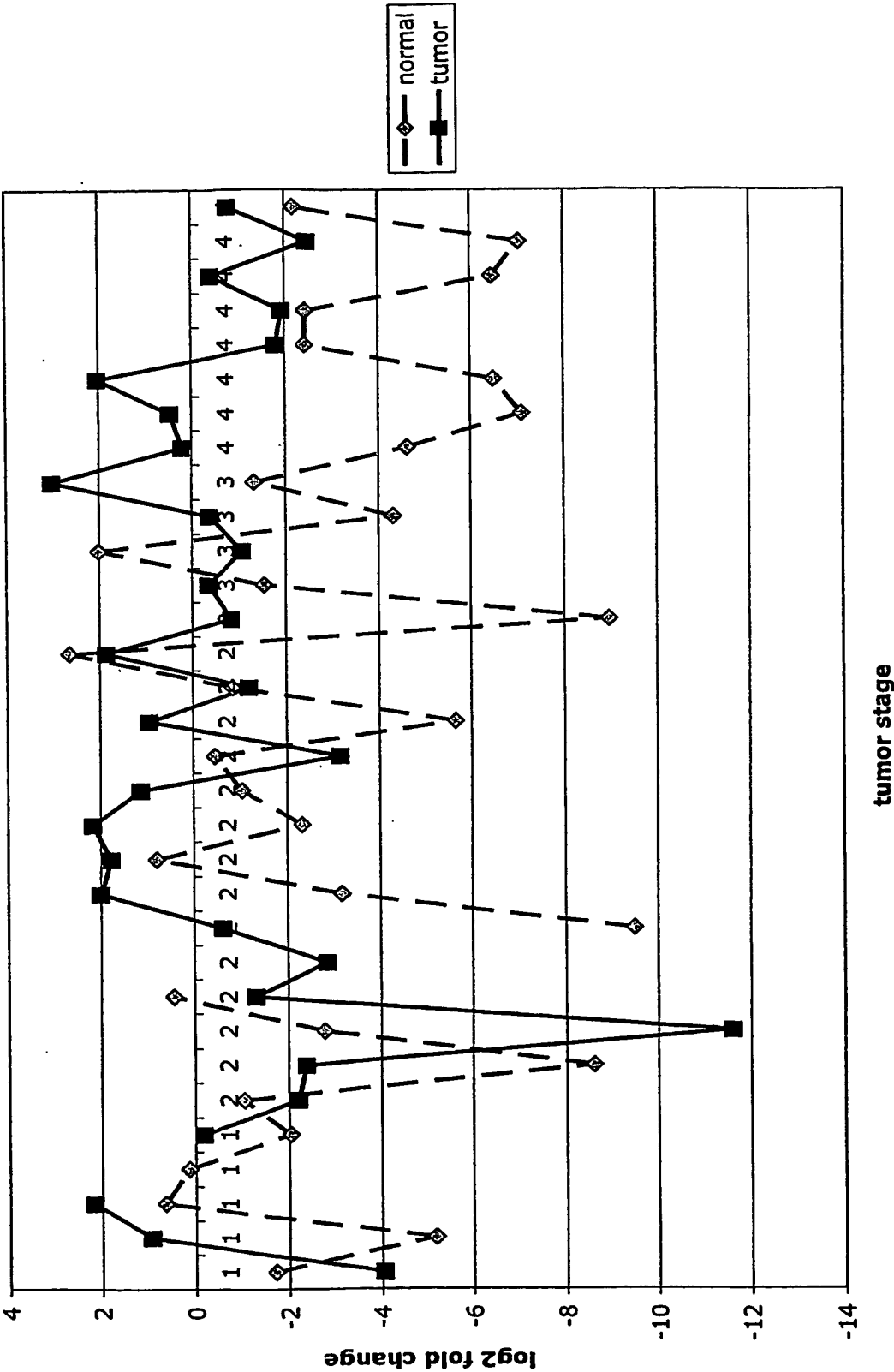


Fig. 10ab SERPINB5



**Fig. 10ac TGFb1**

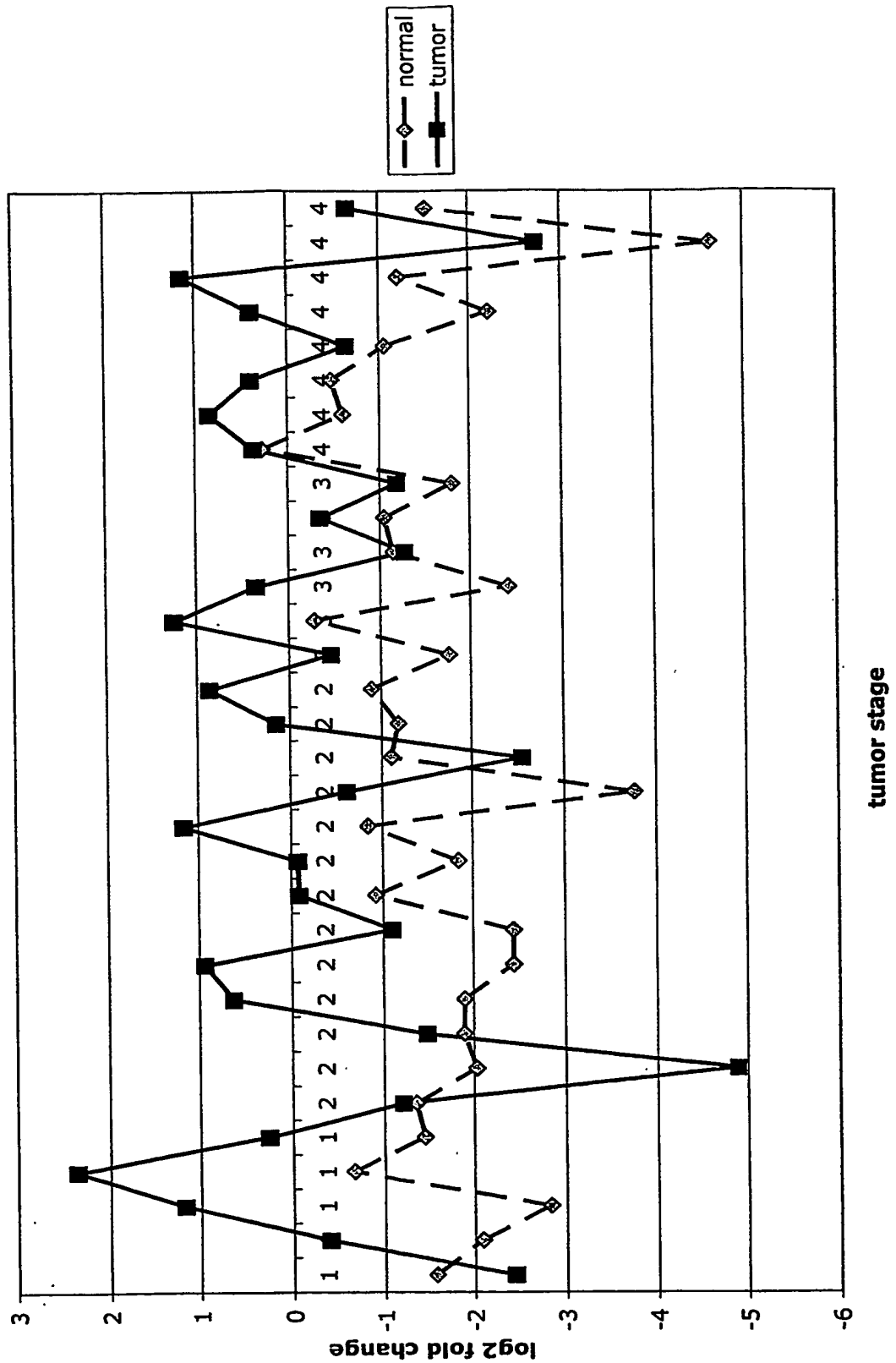
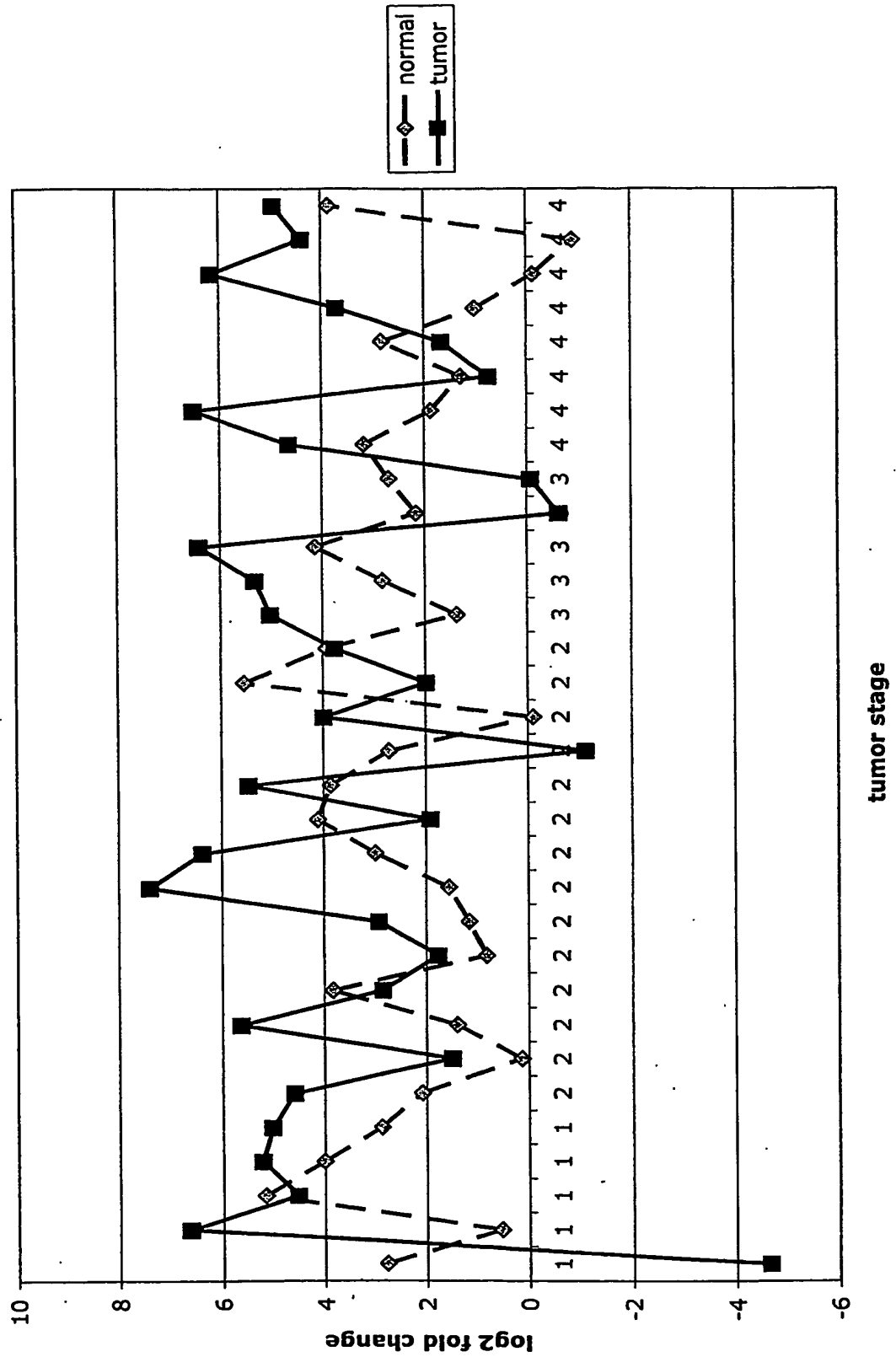


Fig. 10ad CEA



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Fig. 11a Adican

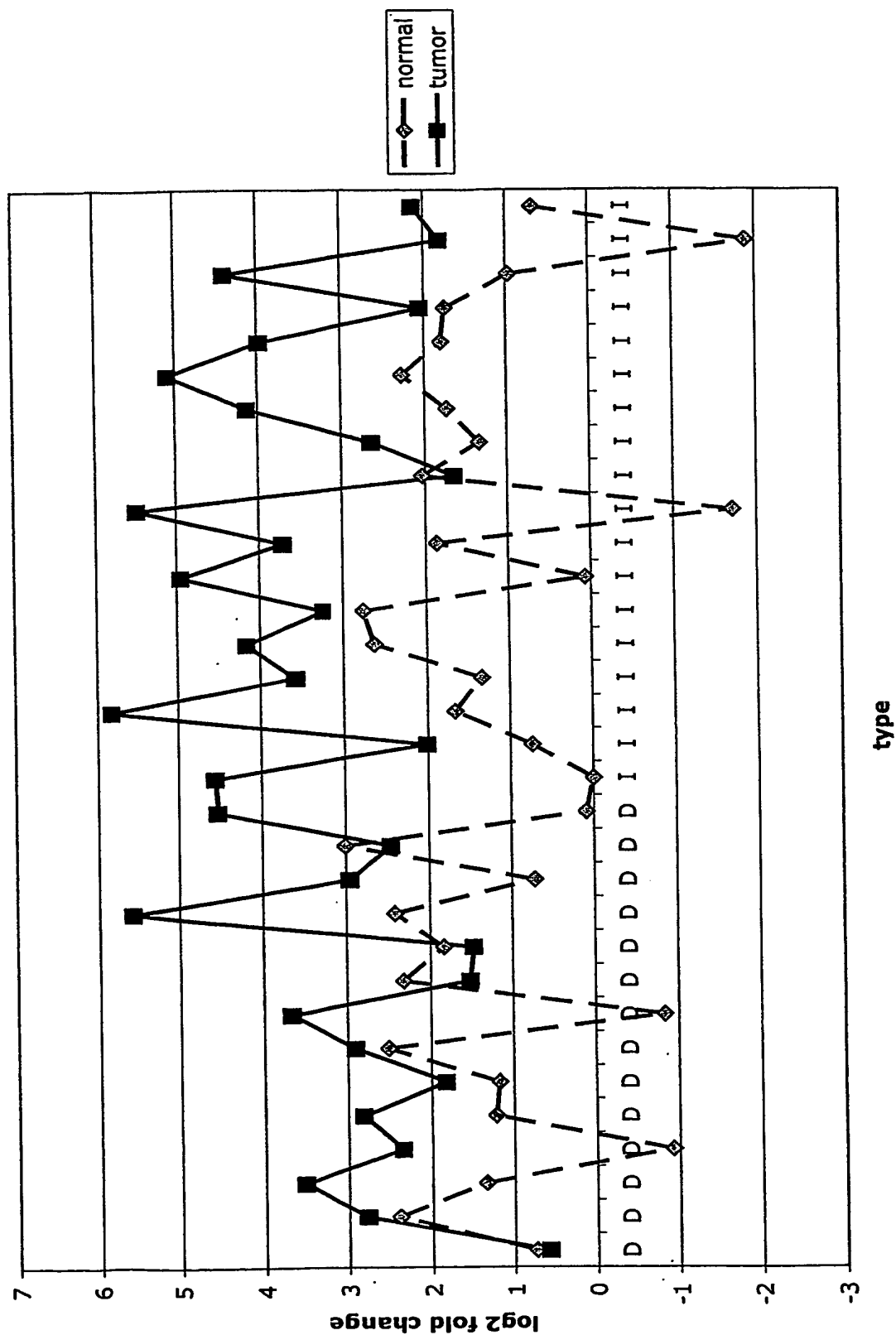


Fig. 11b ASPN

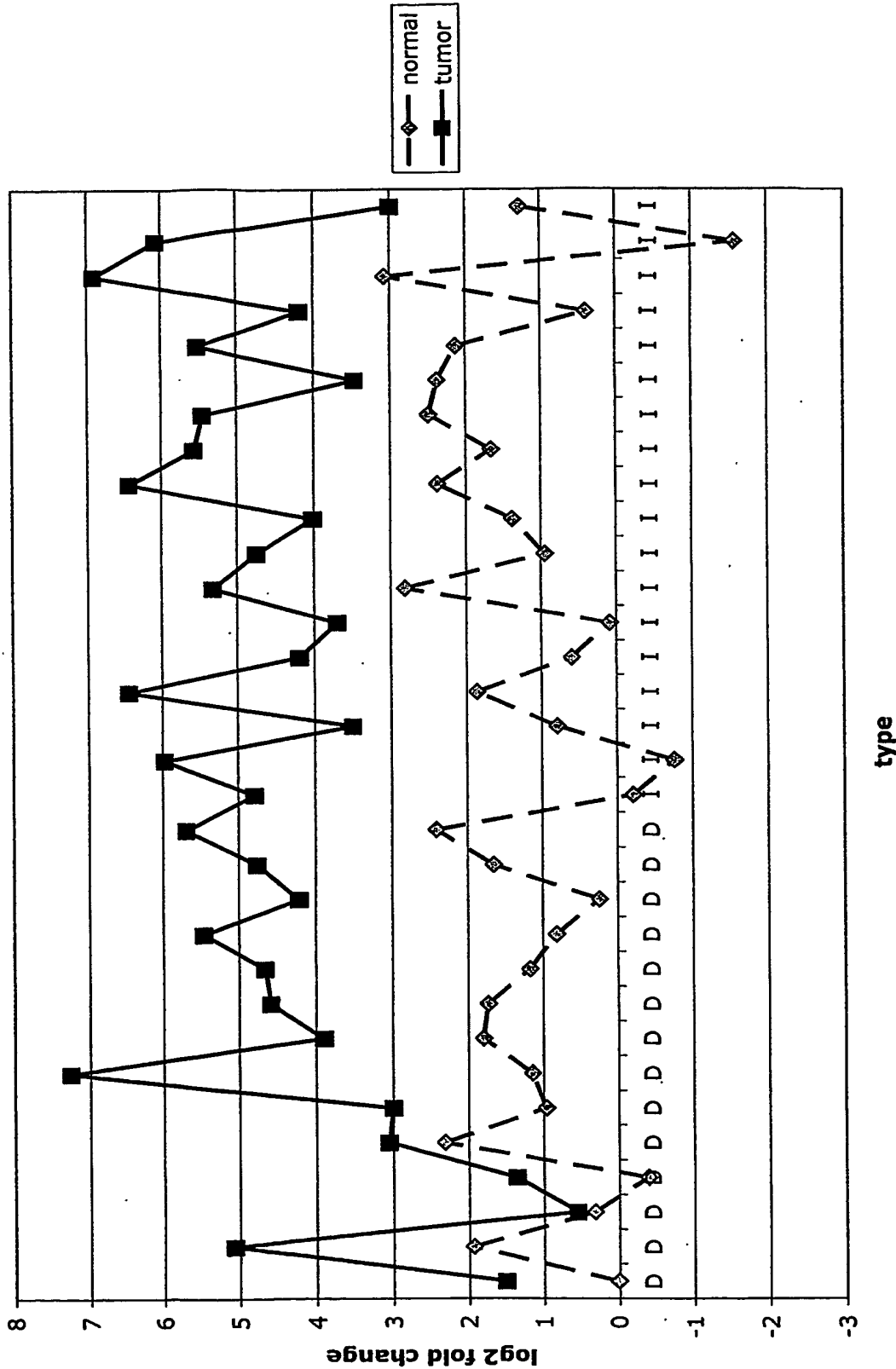


Fig. 11c CSPG2

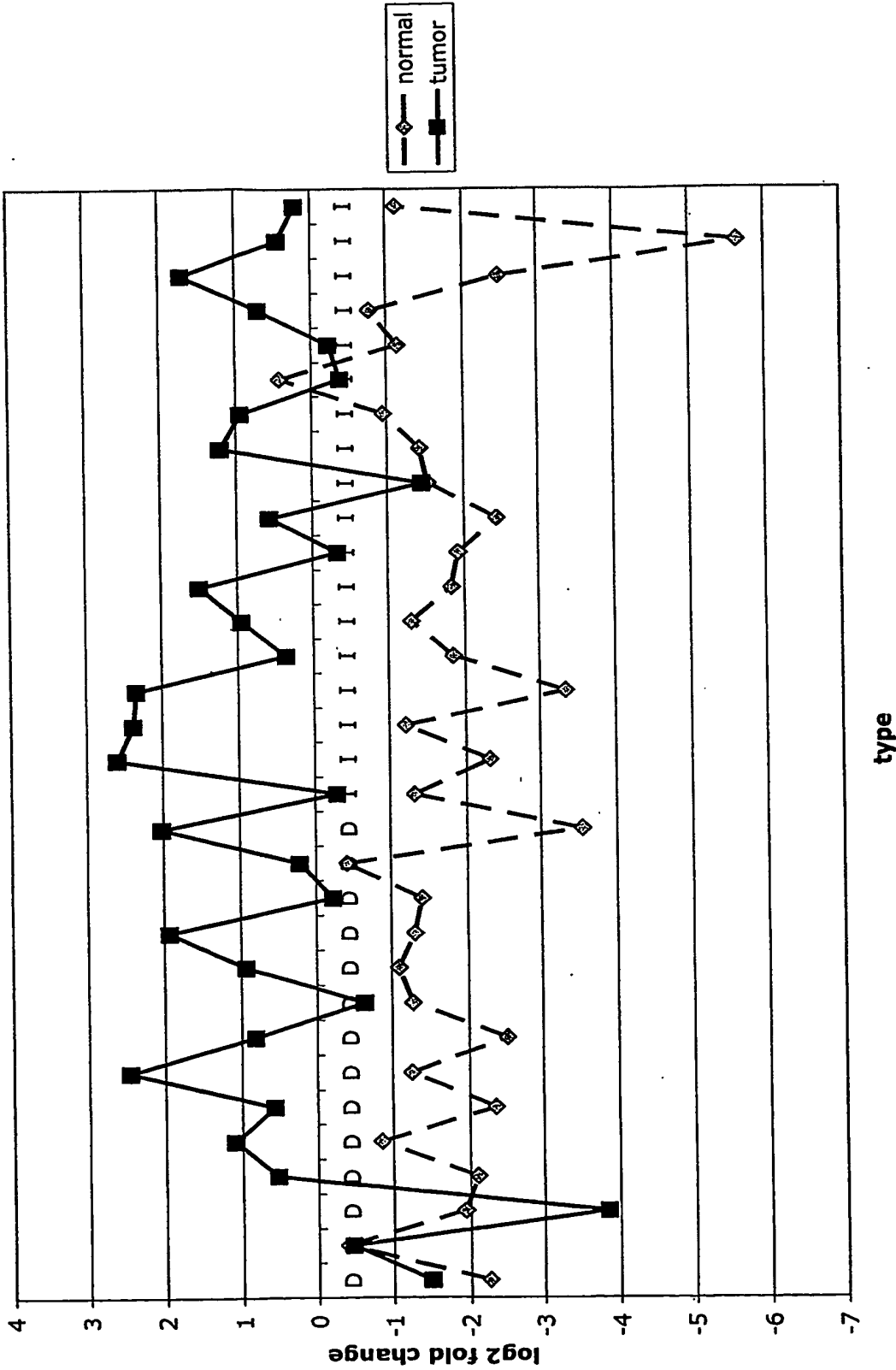




Fig. 11d CST1,2,4

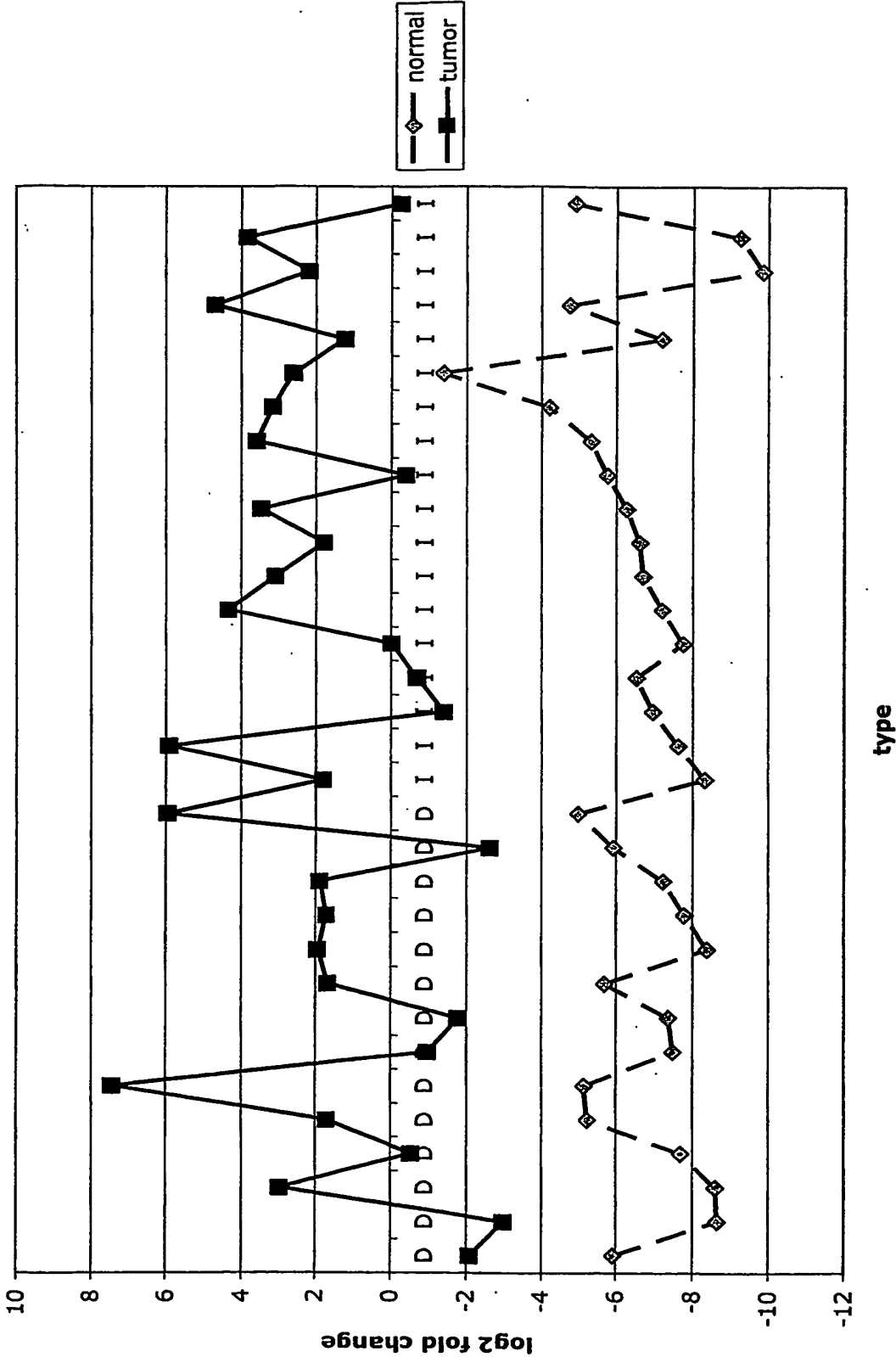


Fig. 11e EFEMP2

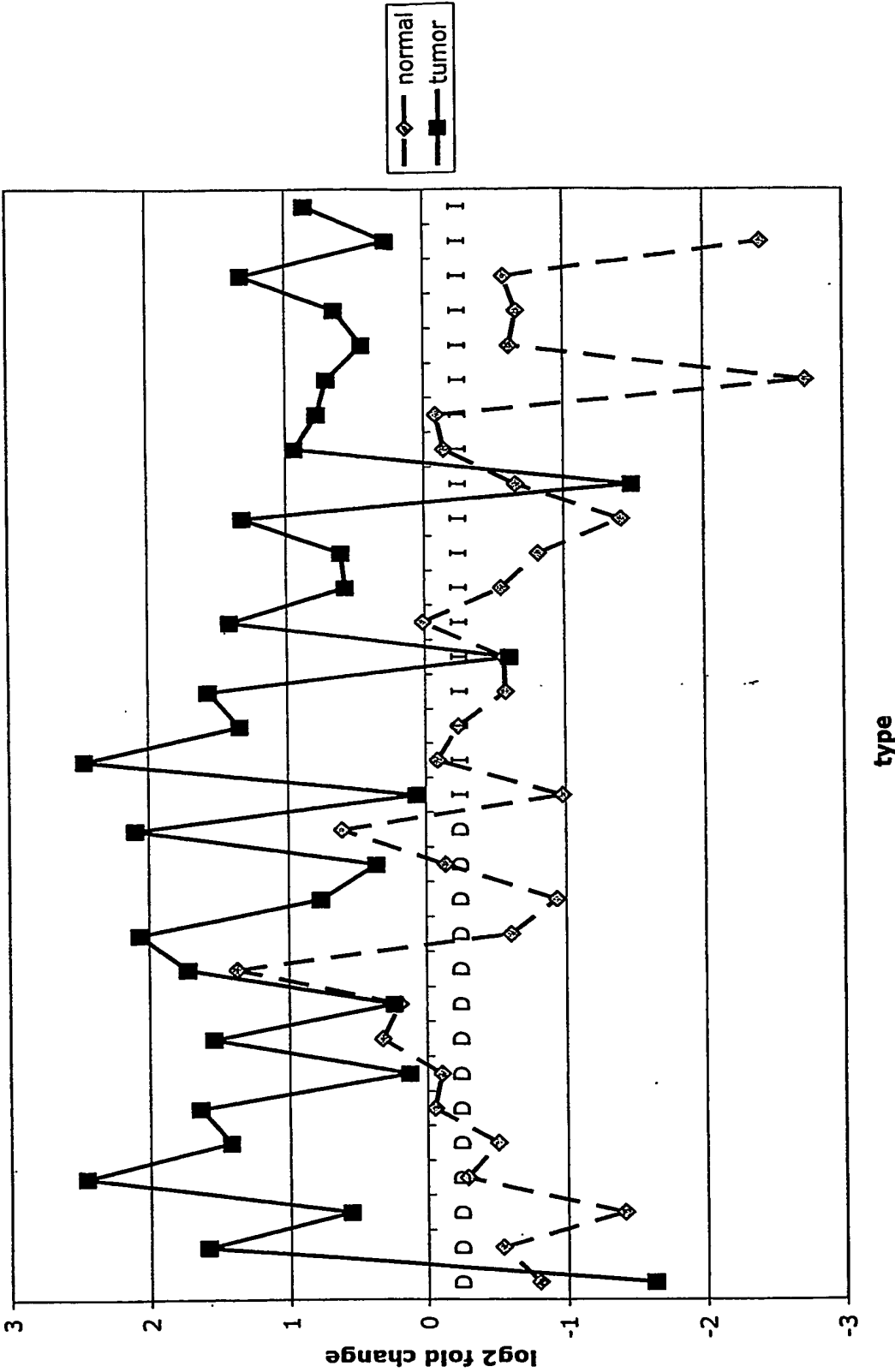


Fig. 11f GGH

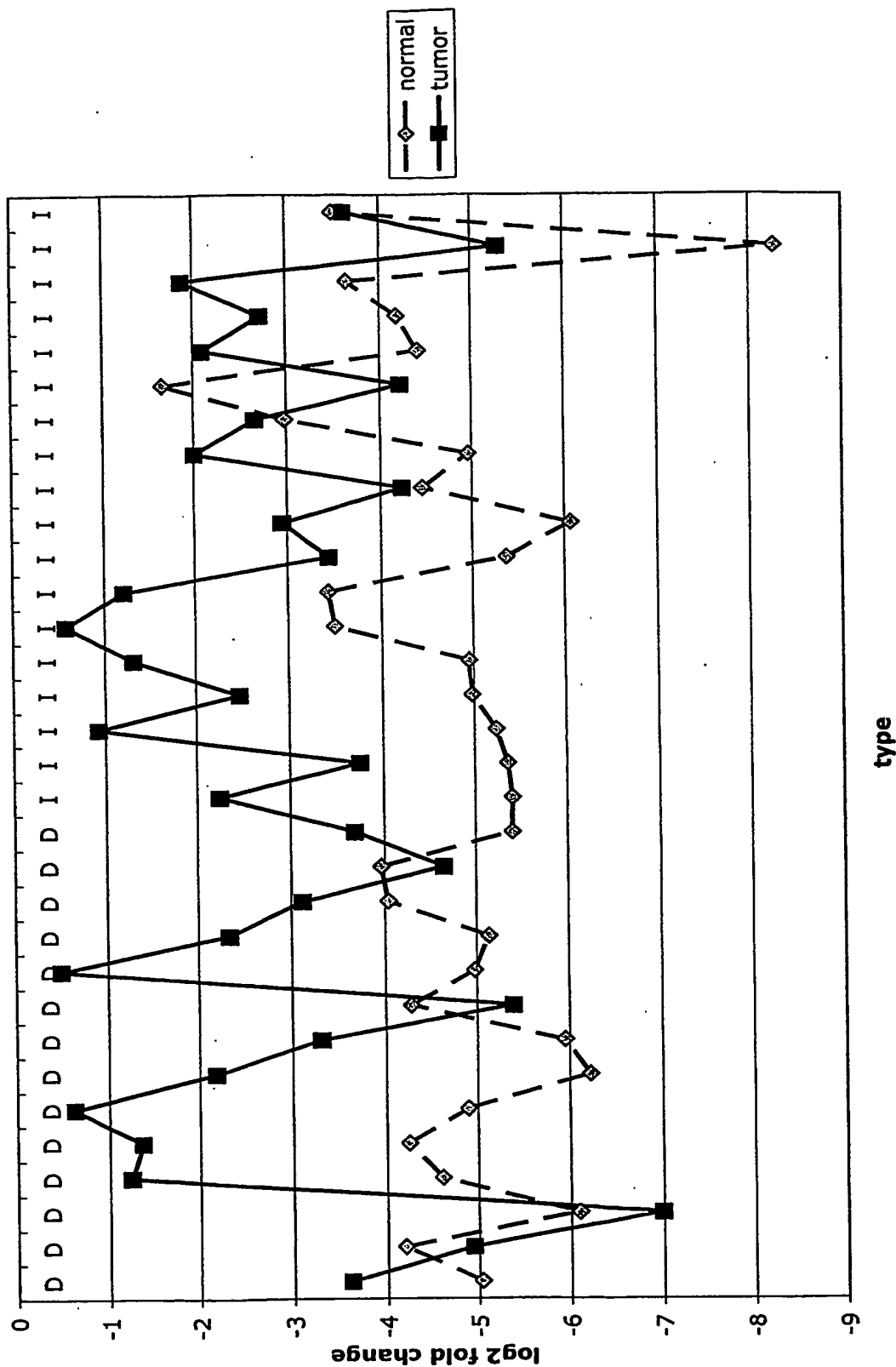


Fig. 11g INHBA

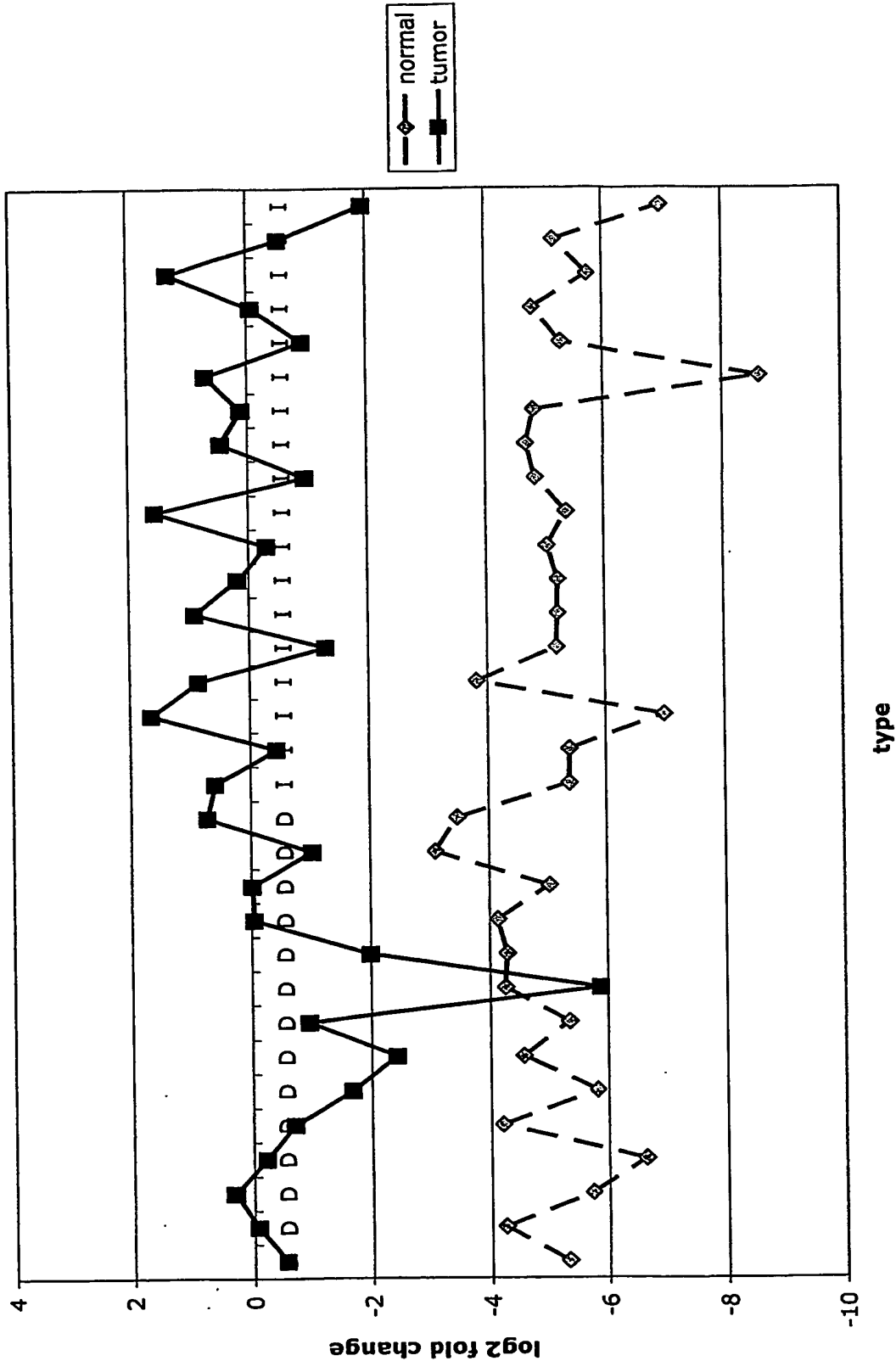


Fig. 11h IGFBP7

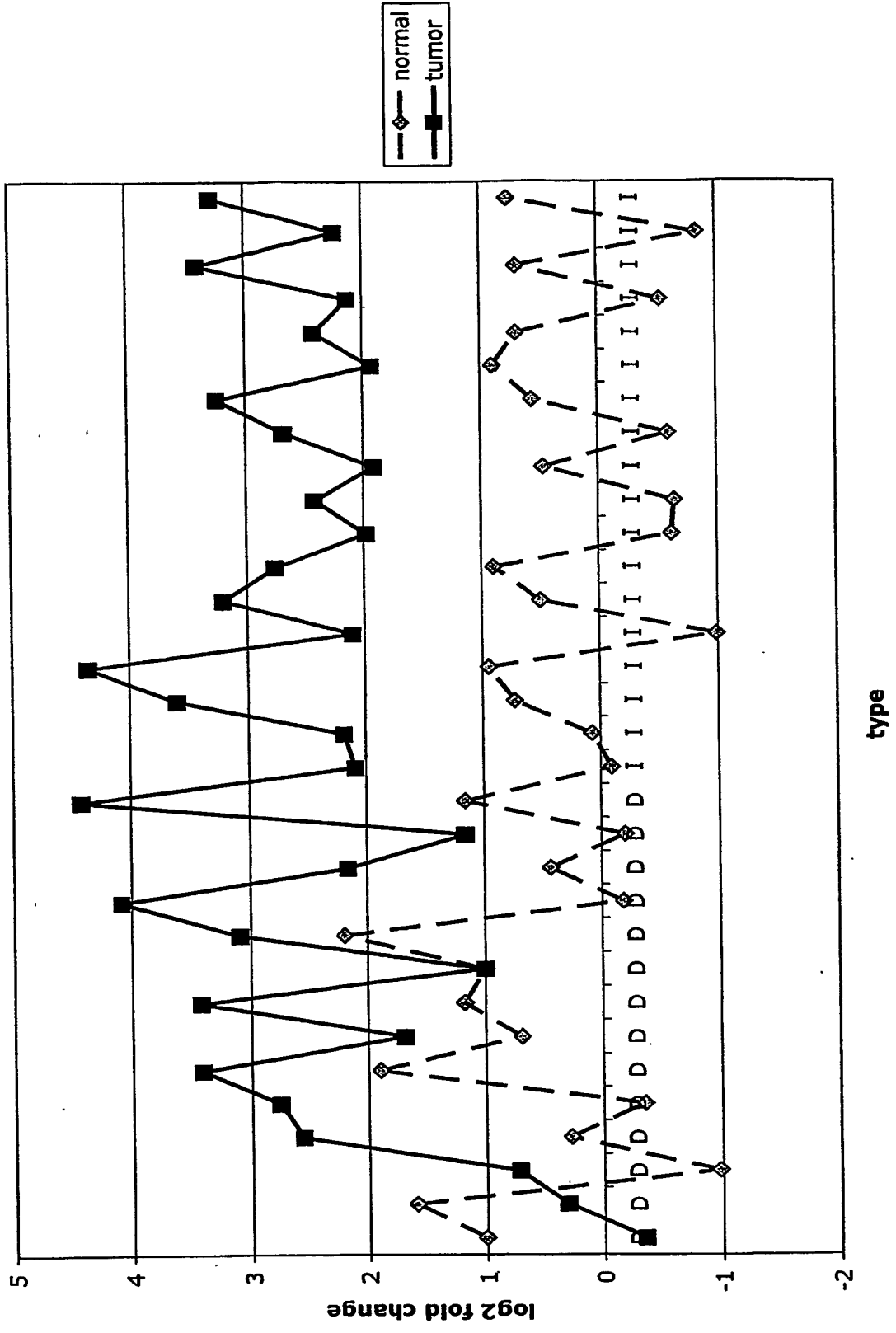


Fig. 11i KLK10

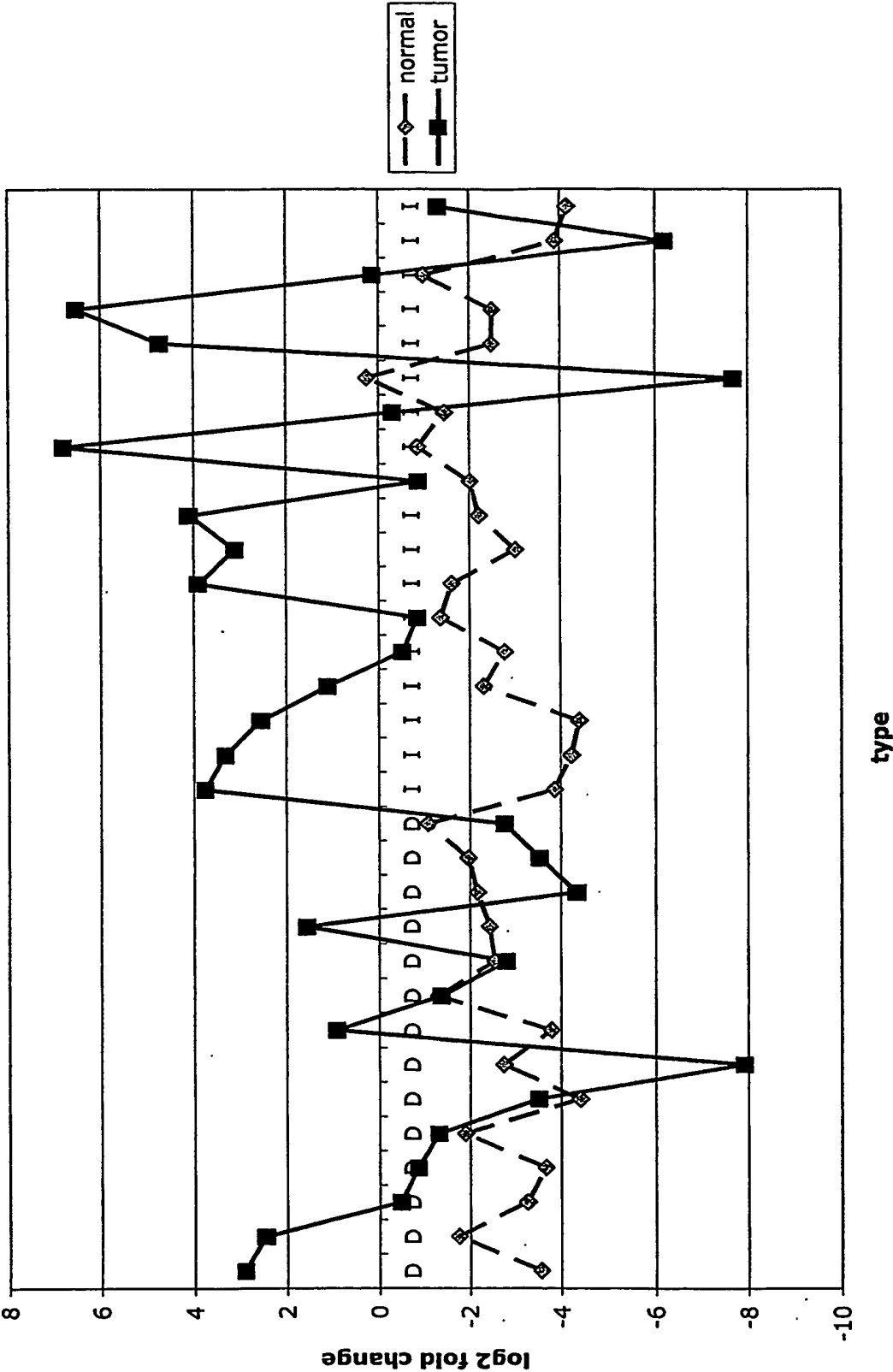


Fig. 11j LEPRE1

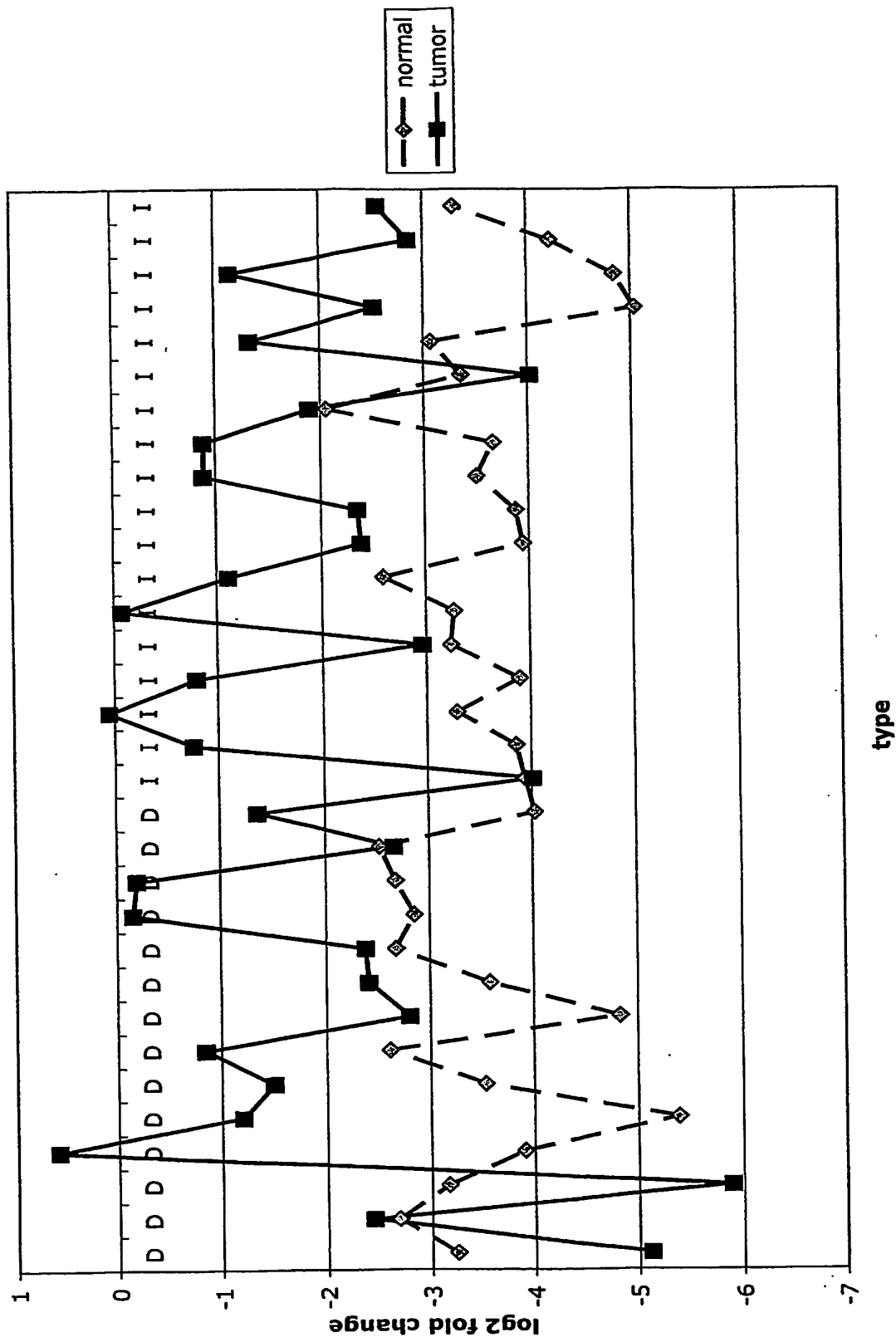


Fig. 11k LUM

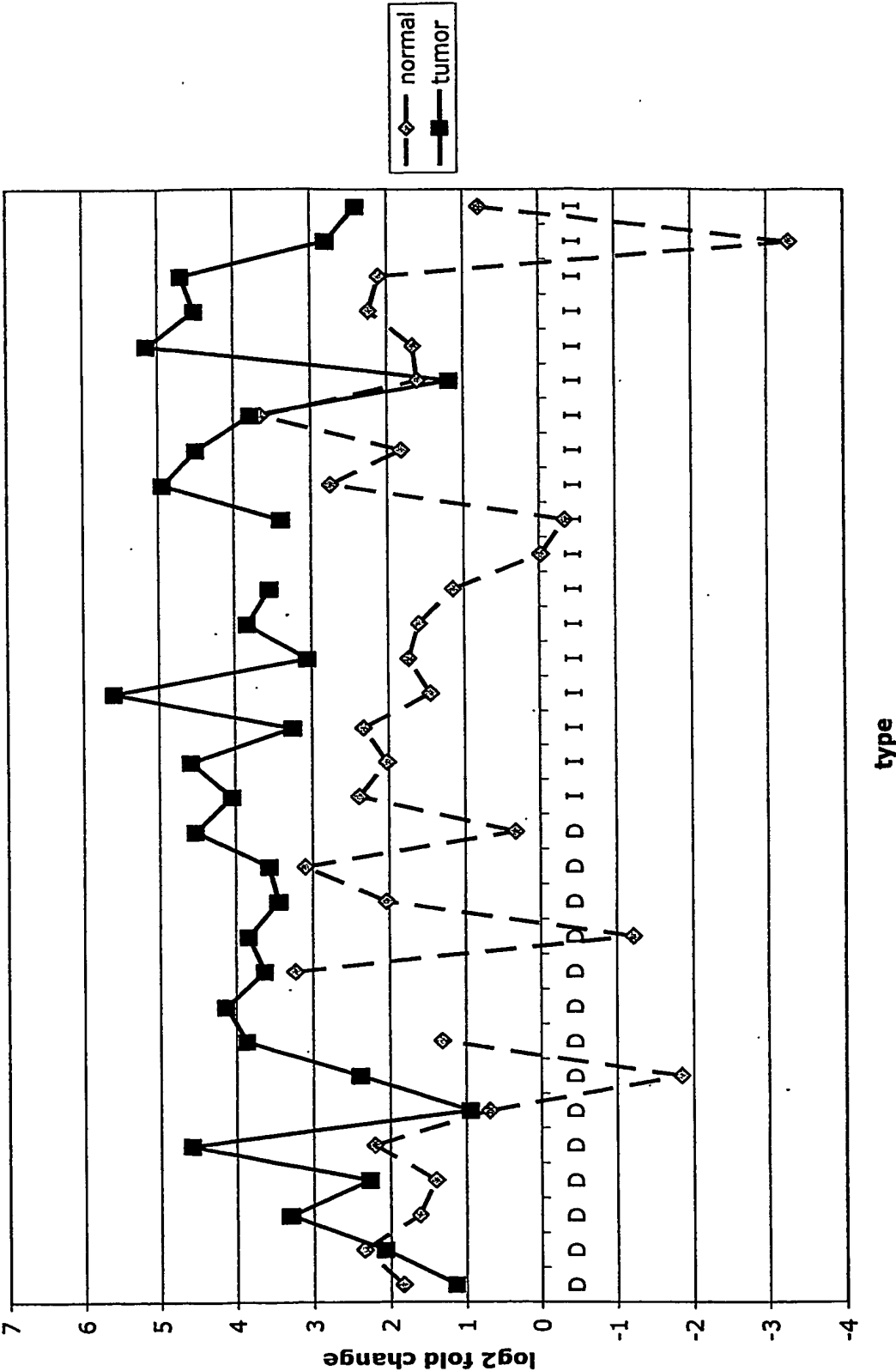




Fig. 11I LOXL2

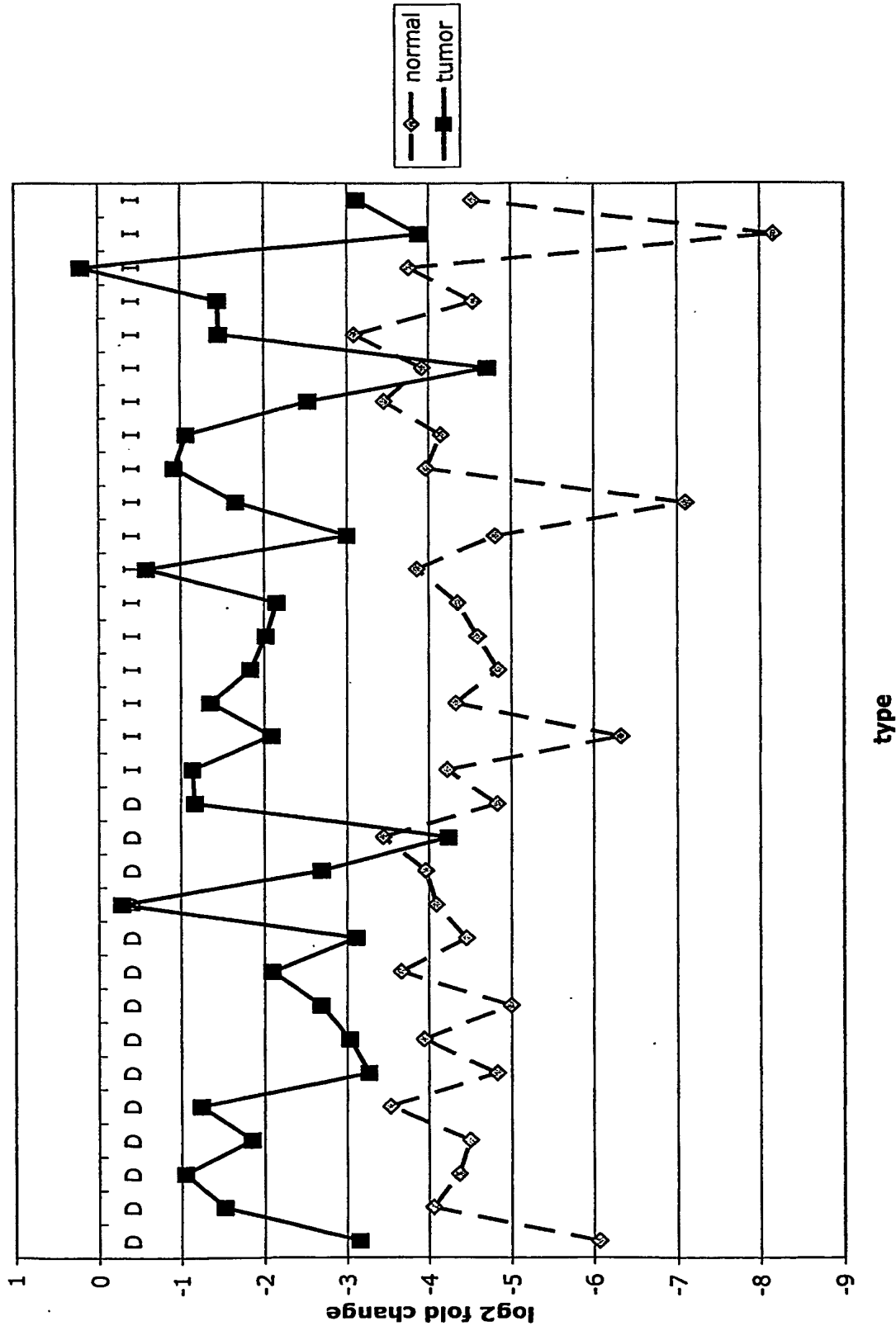


Fig. 11m MMP12

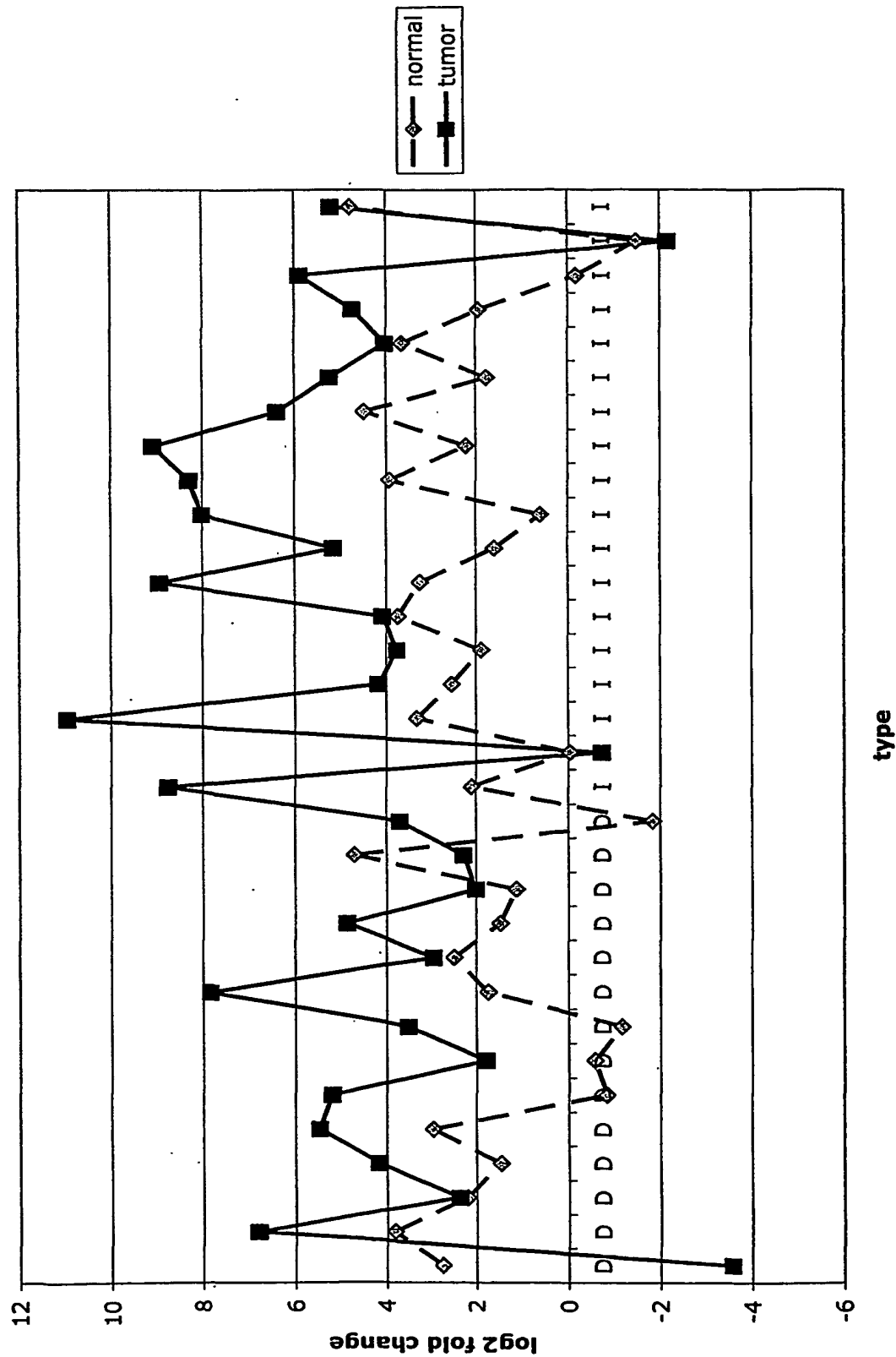


Fig. 11n TIMP1

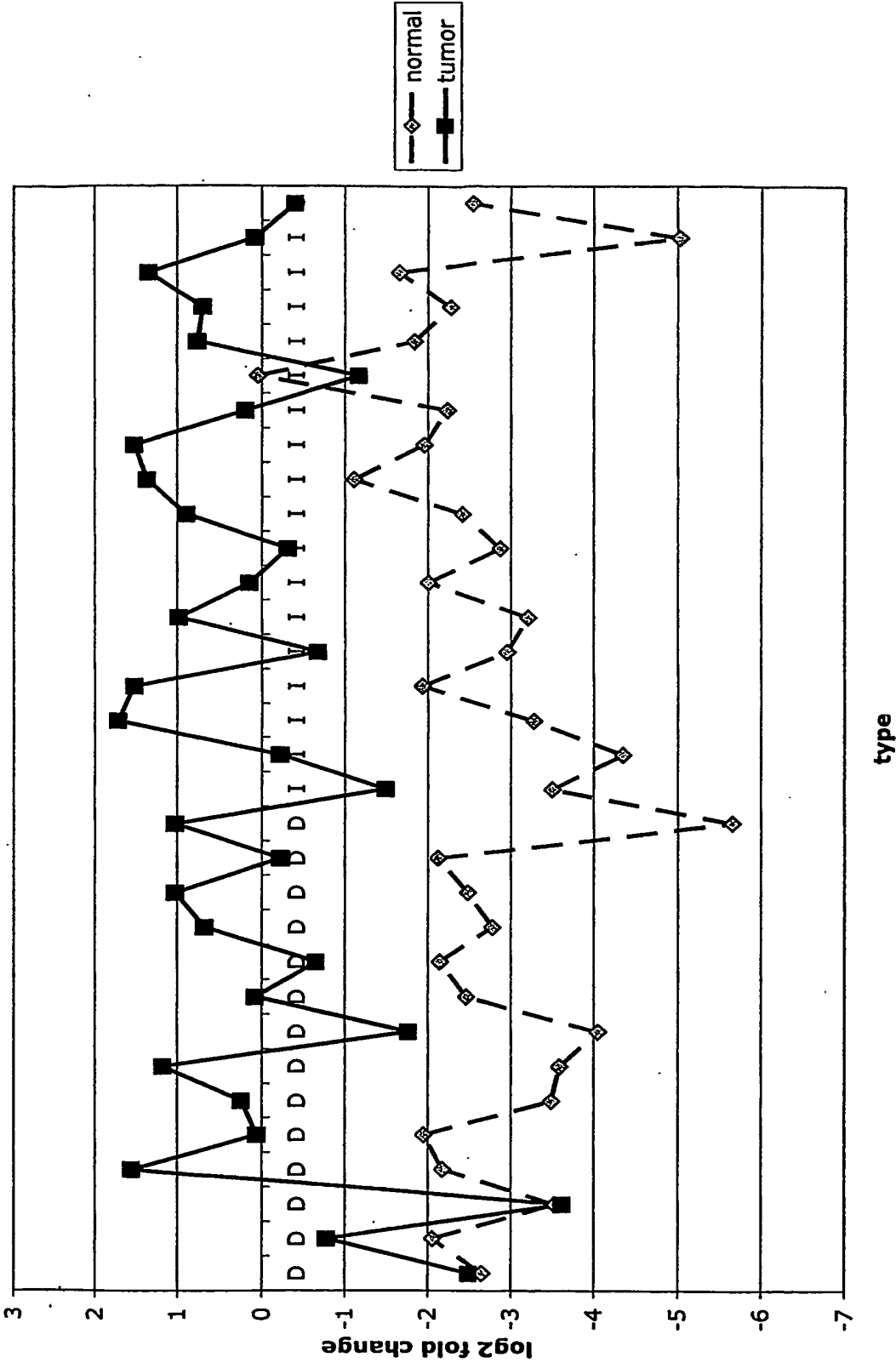


Fig. 11o ASAH1

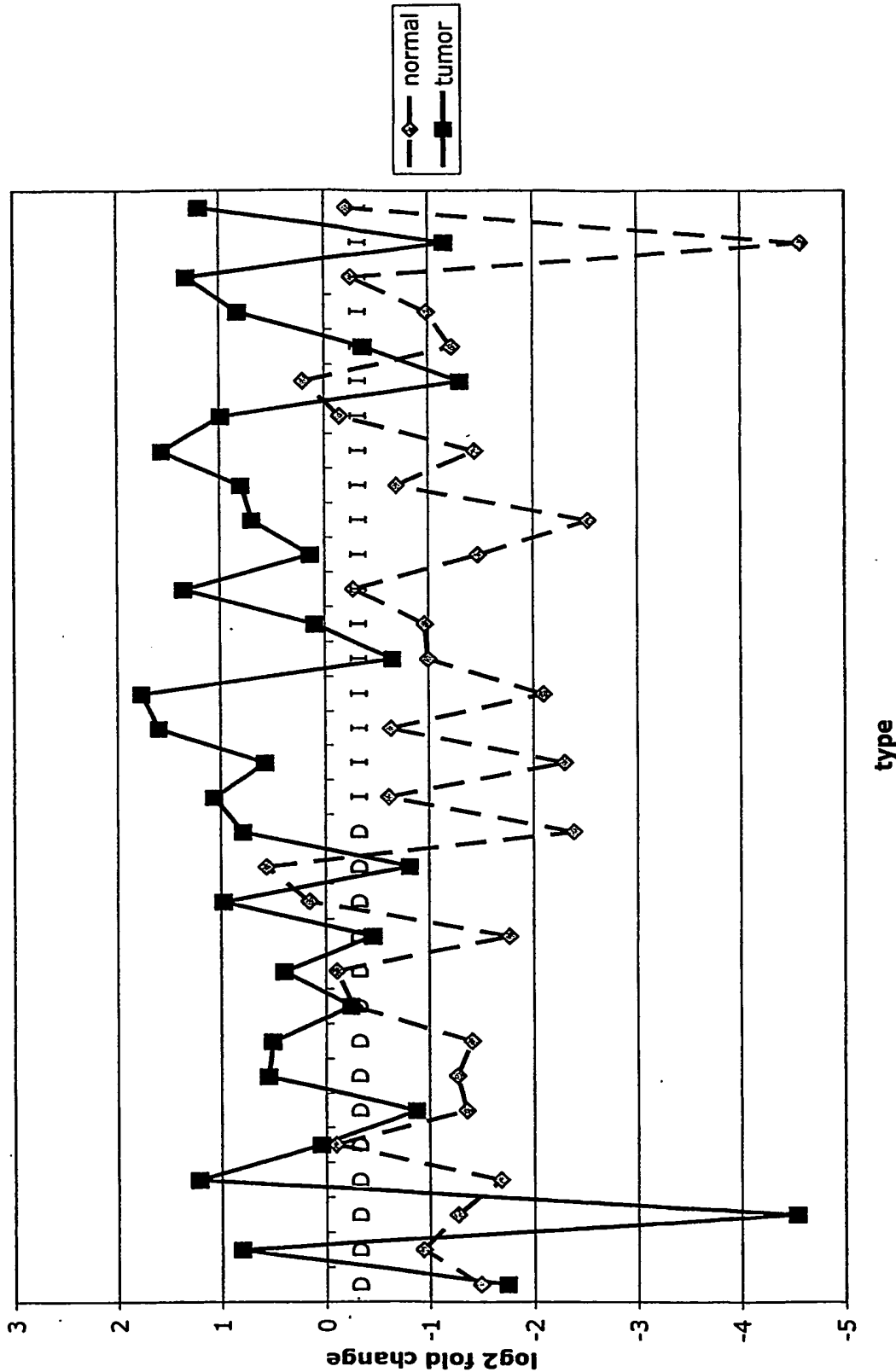
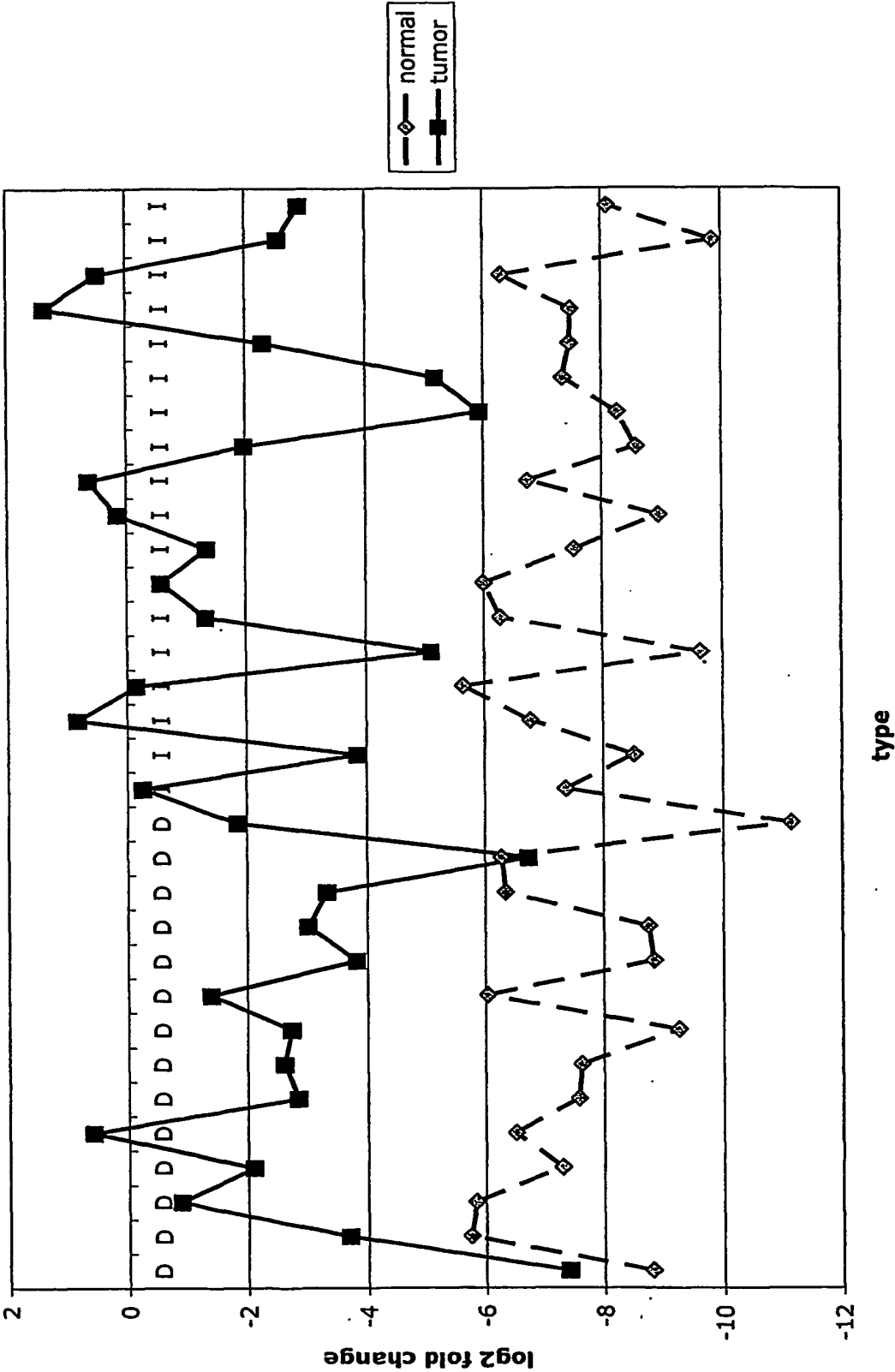


Fig. 11p SPP1



**Fig. 11q SFRP2**

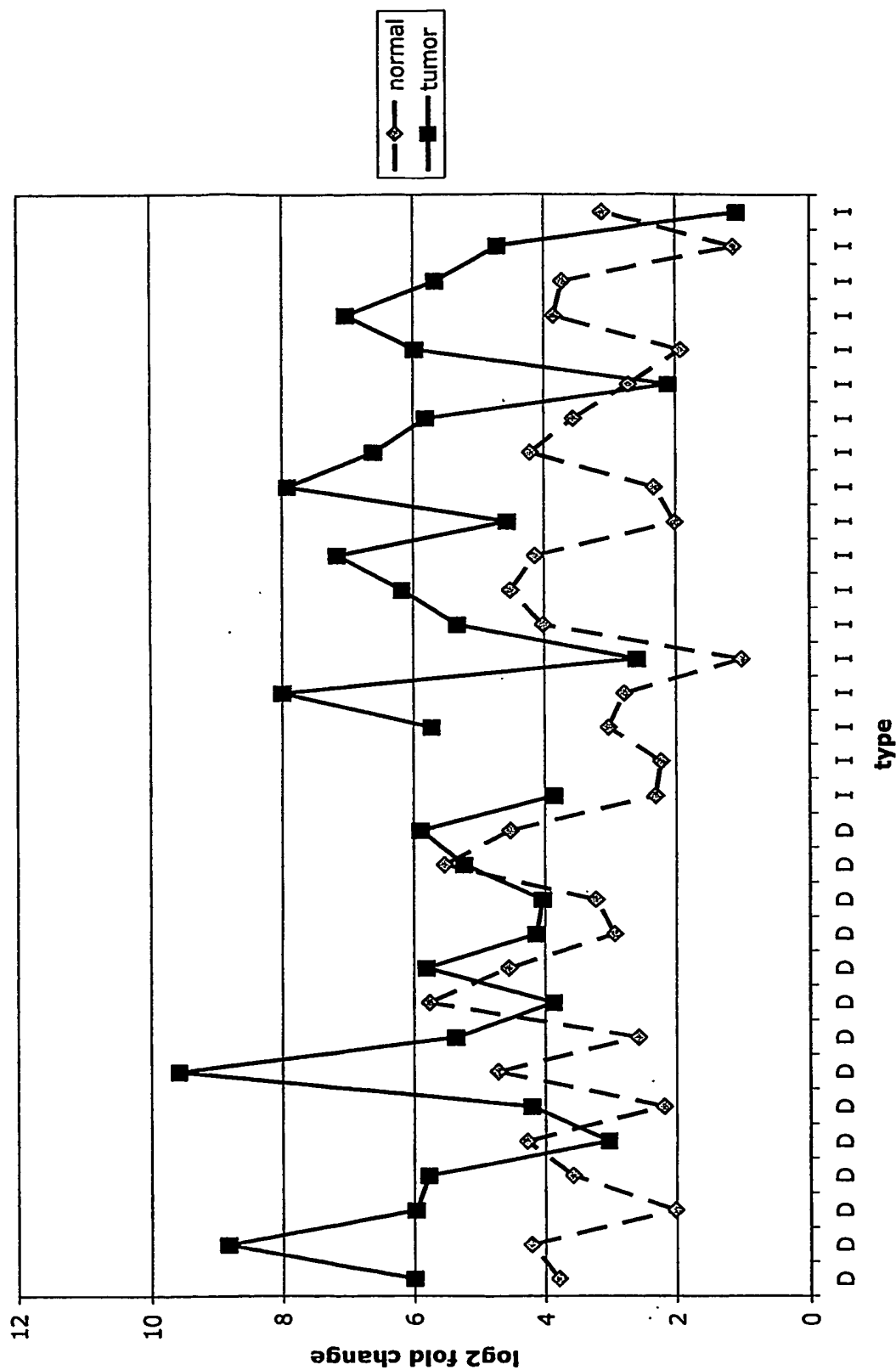


Fig. 11r SFRP4

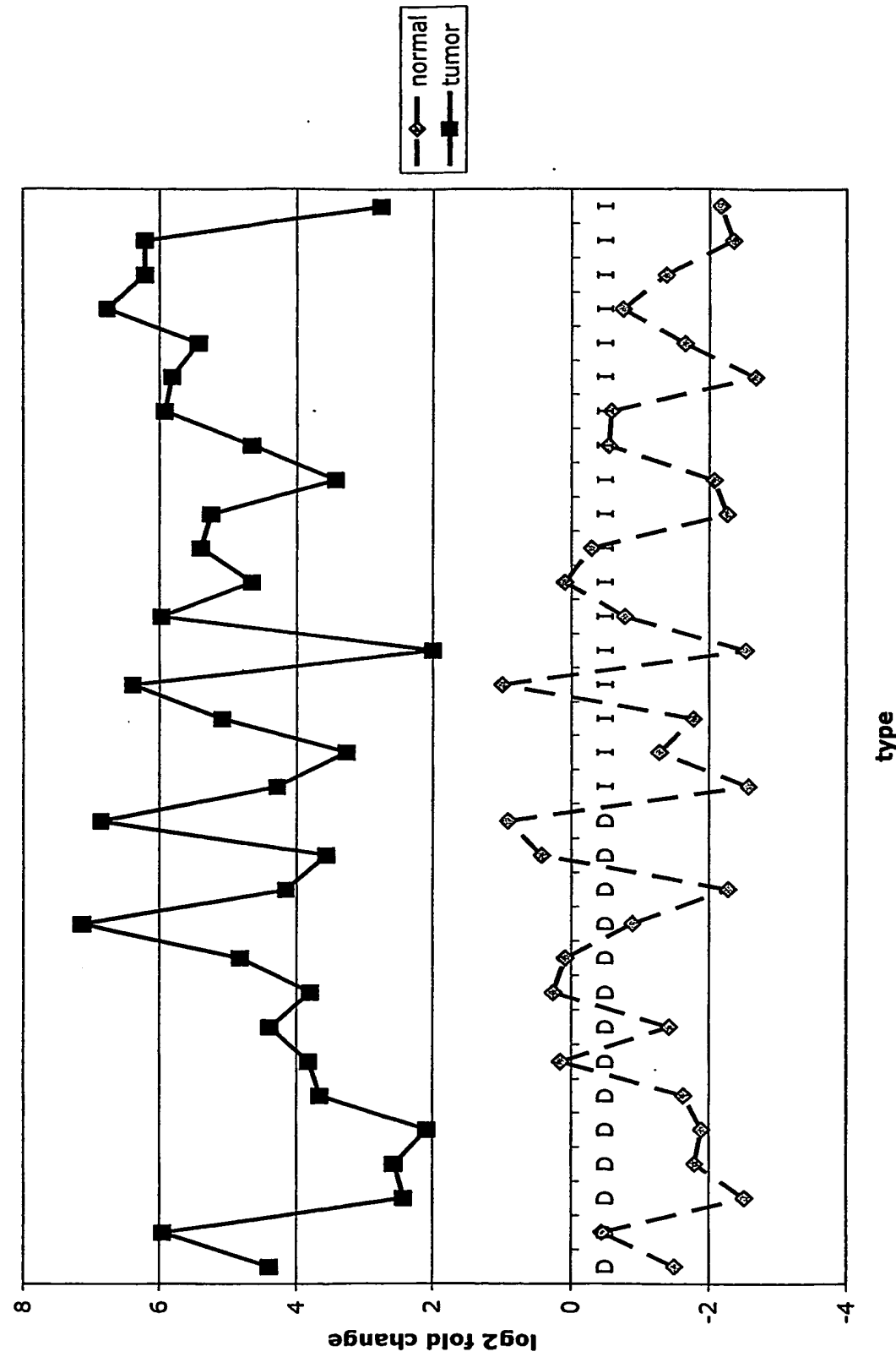


Fig. 11s SPARC

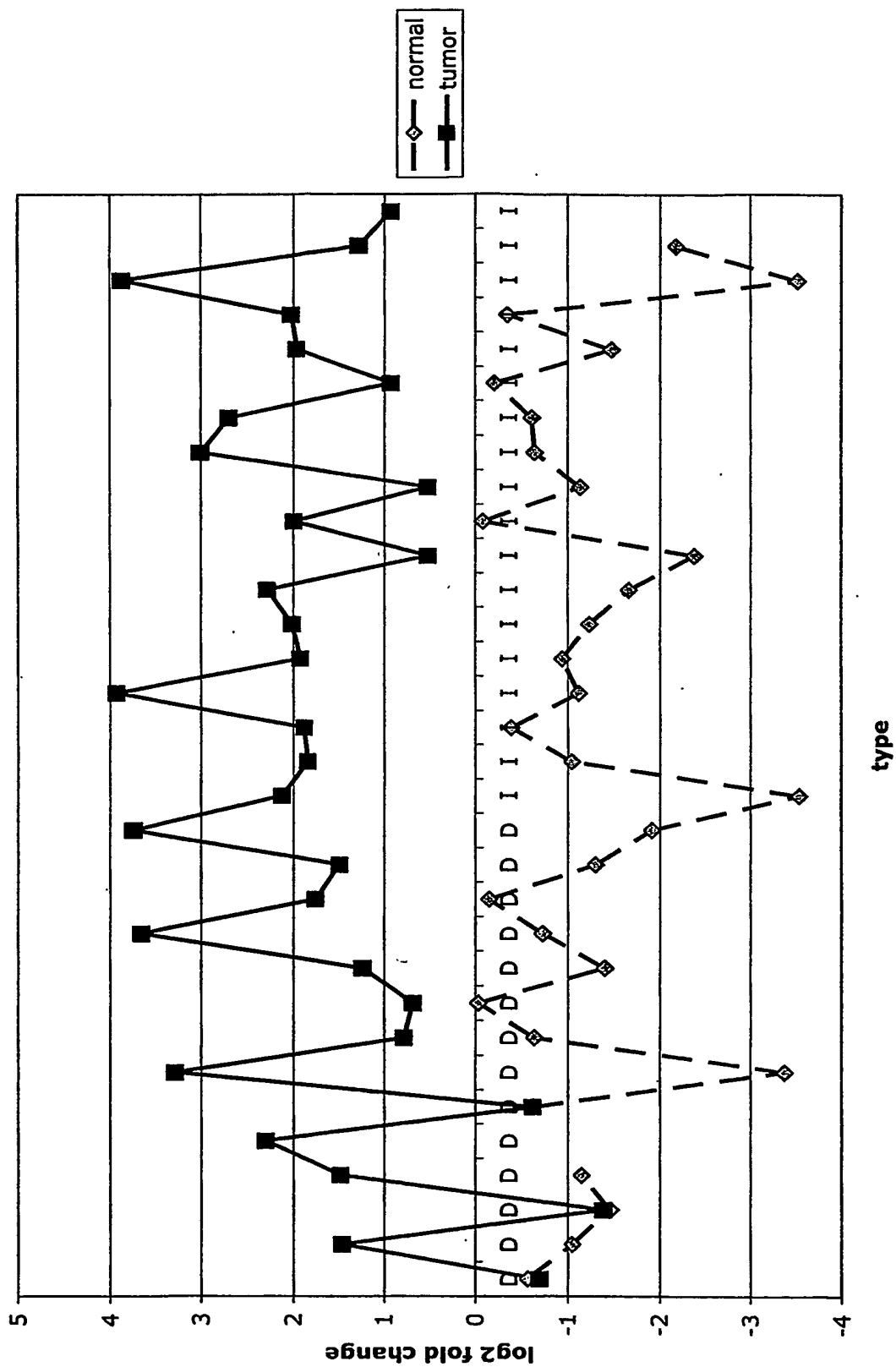
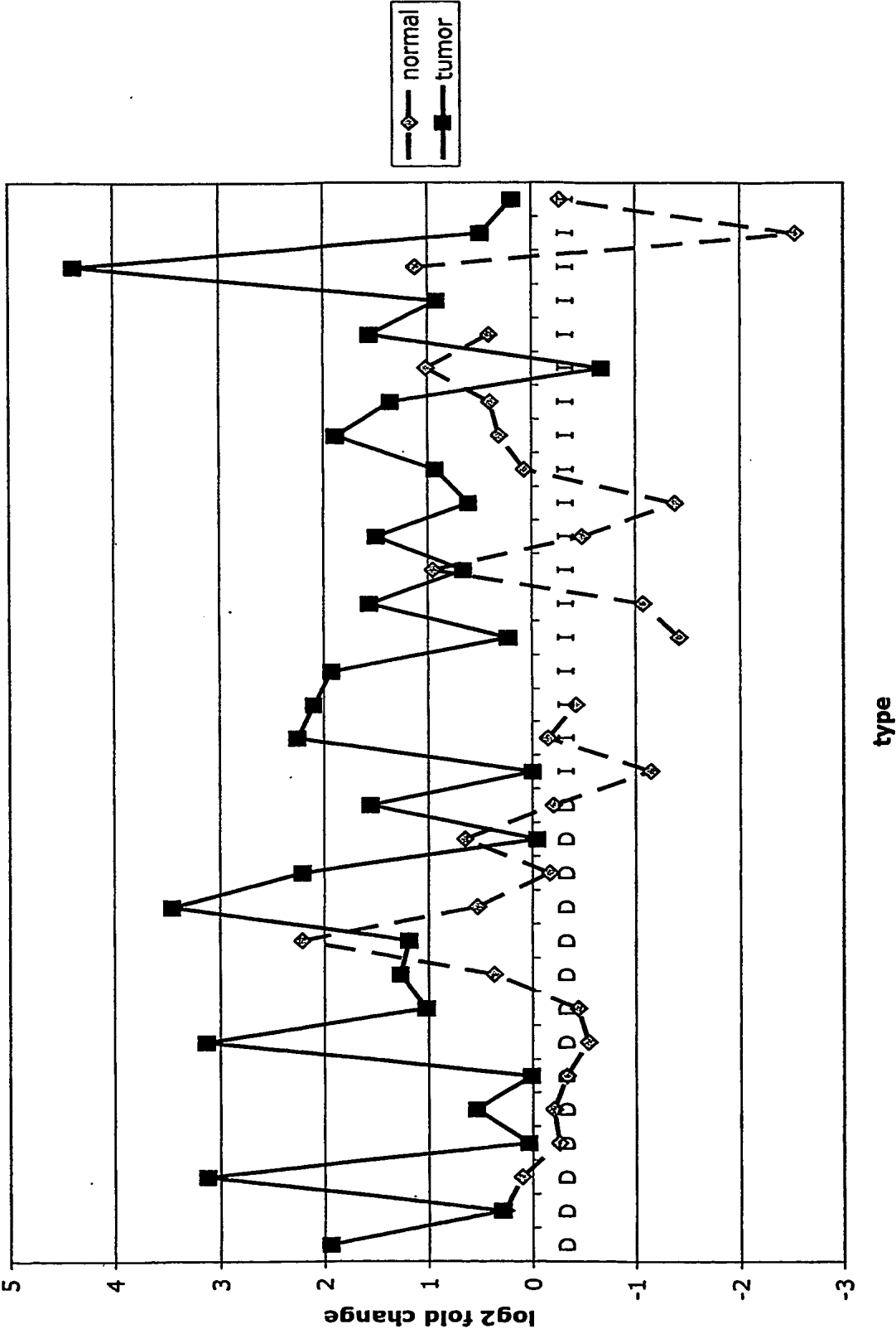




Fig. 11t PRSS11



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Fig. 11u THBS2

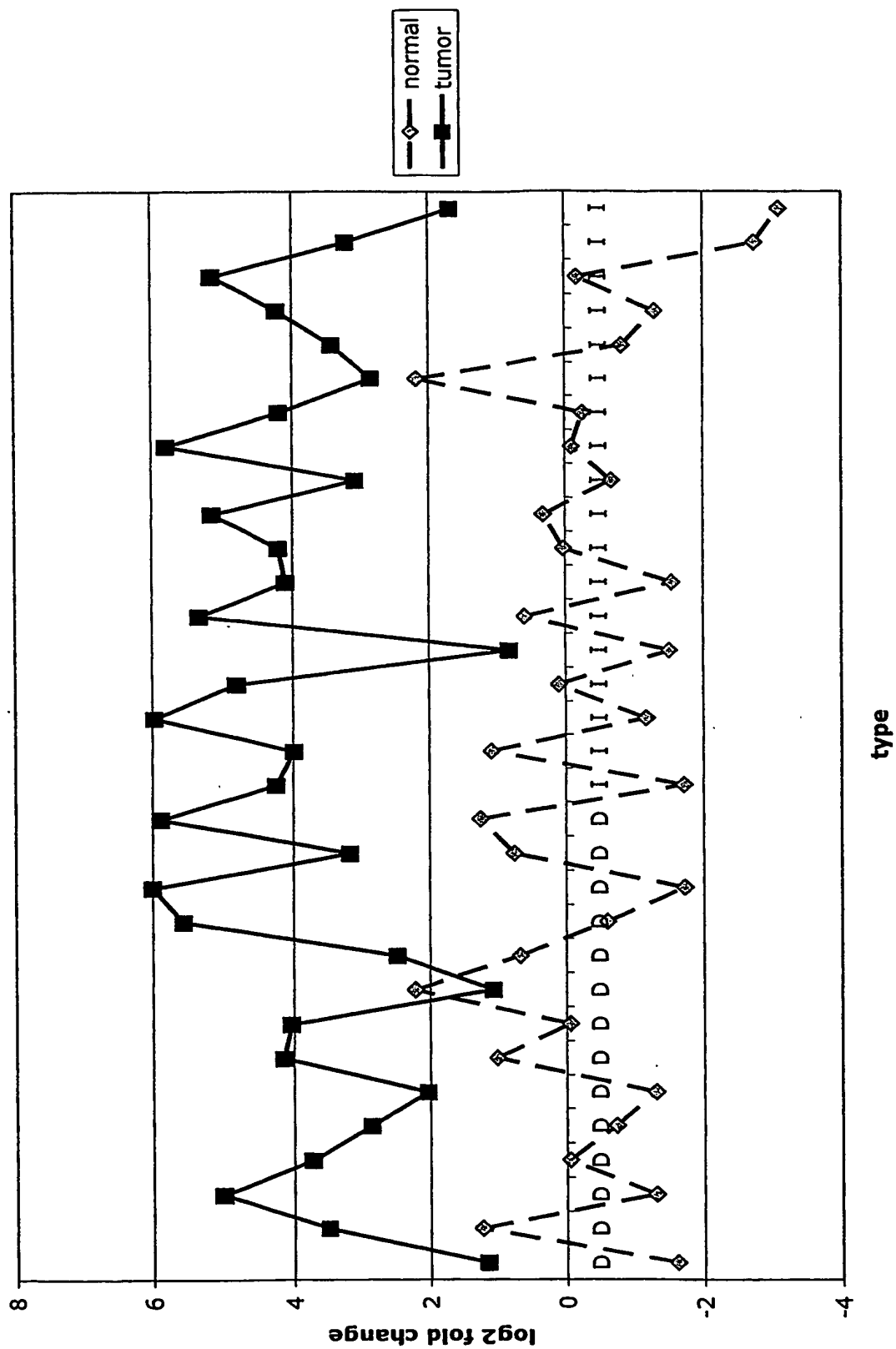


Fig. 11v TG

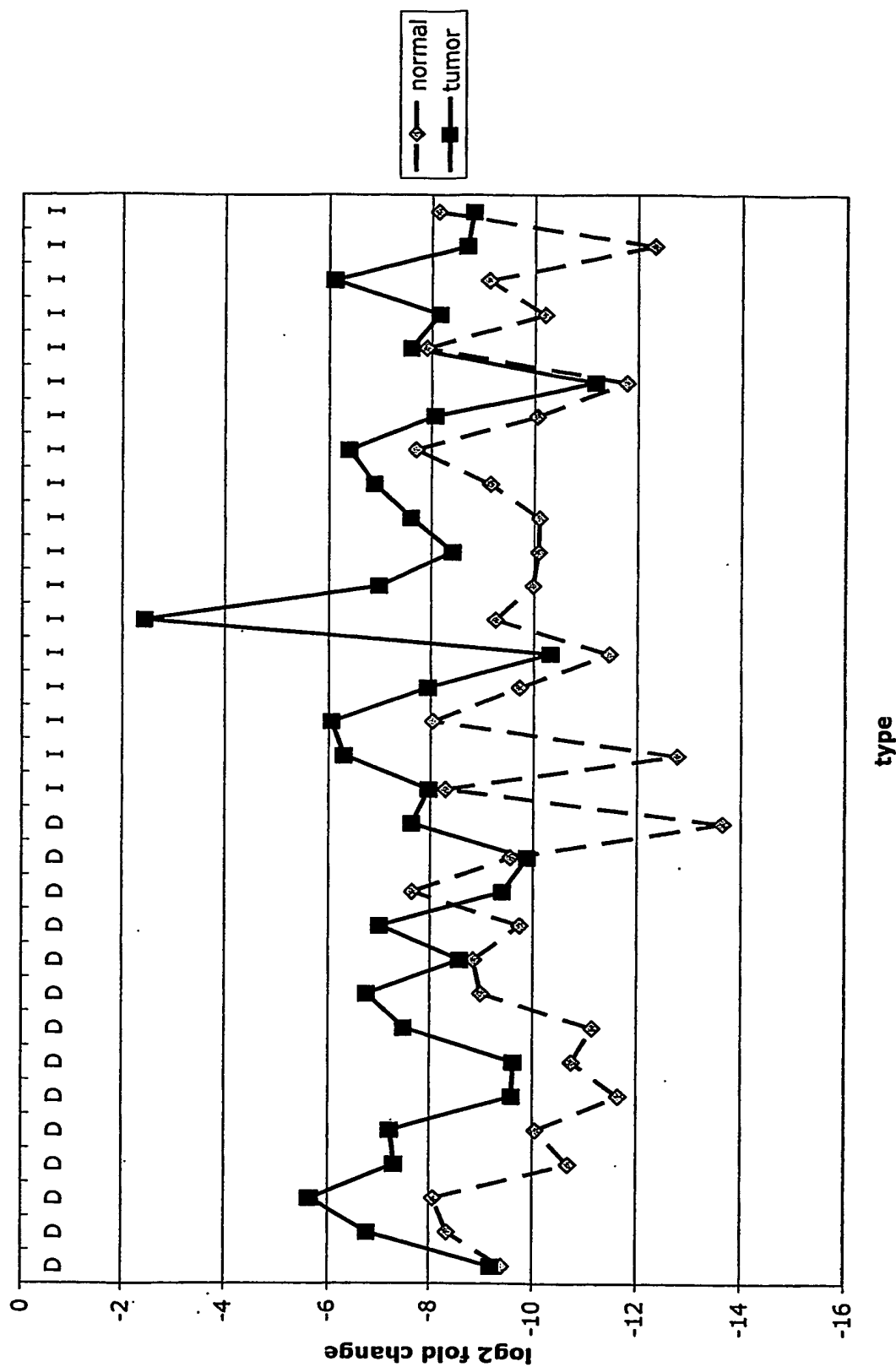
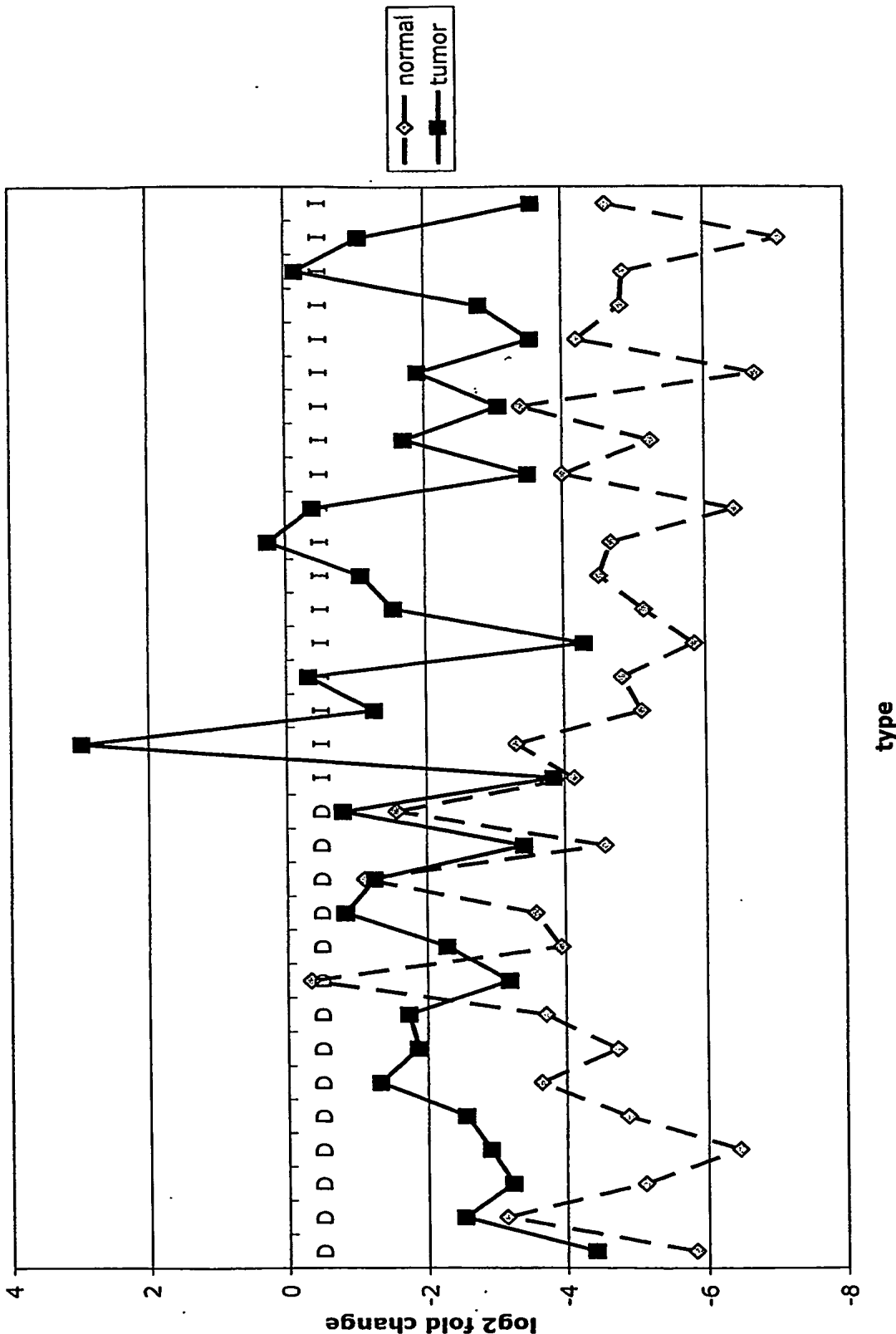


Fig. 11W TGFBI



**Fig. 11x CGR11**

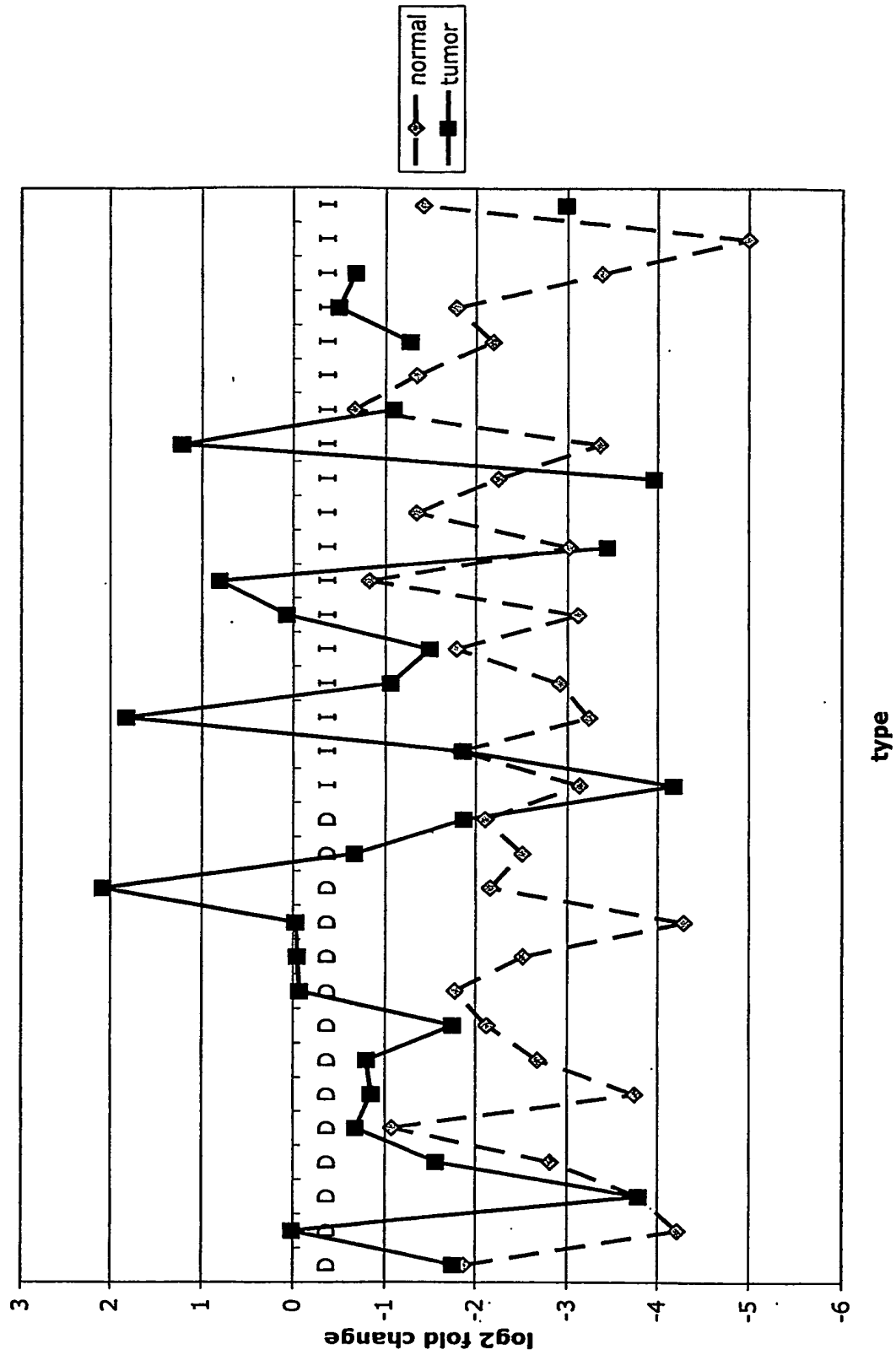


Fig. 11y SERPINH1

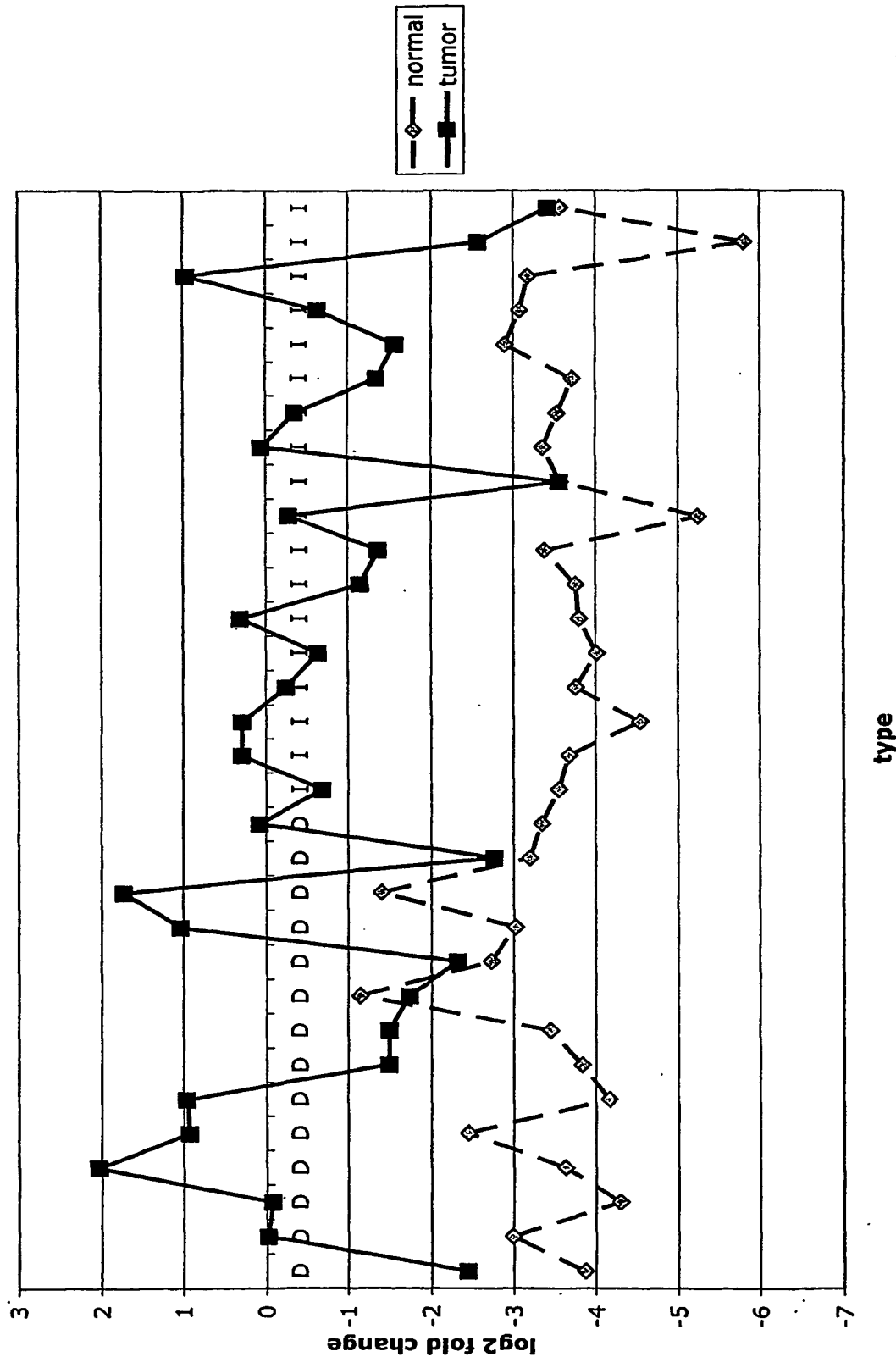


Fig. 11z MMP2

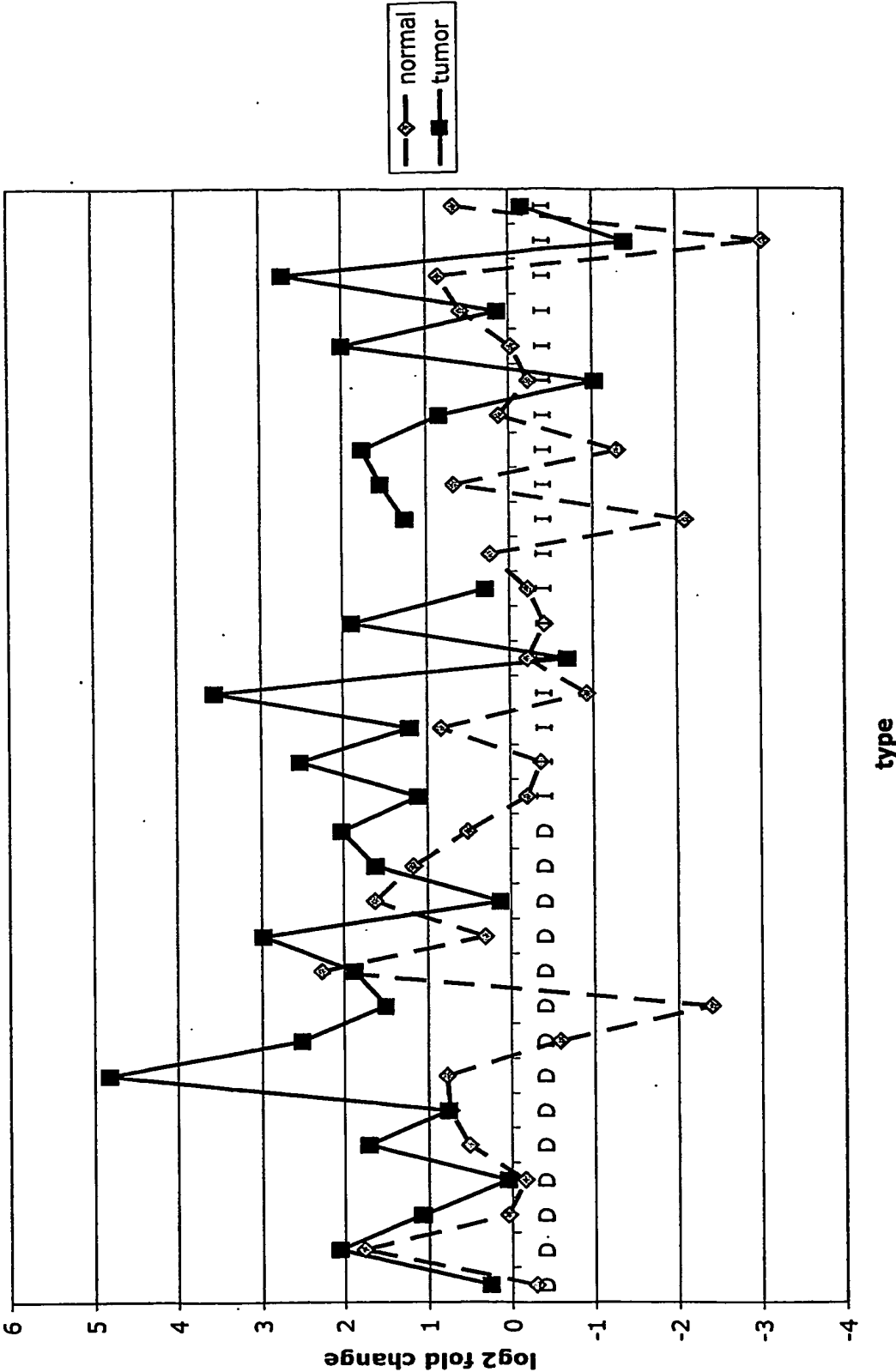


Fig. 11aa PCSK5

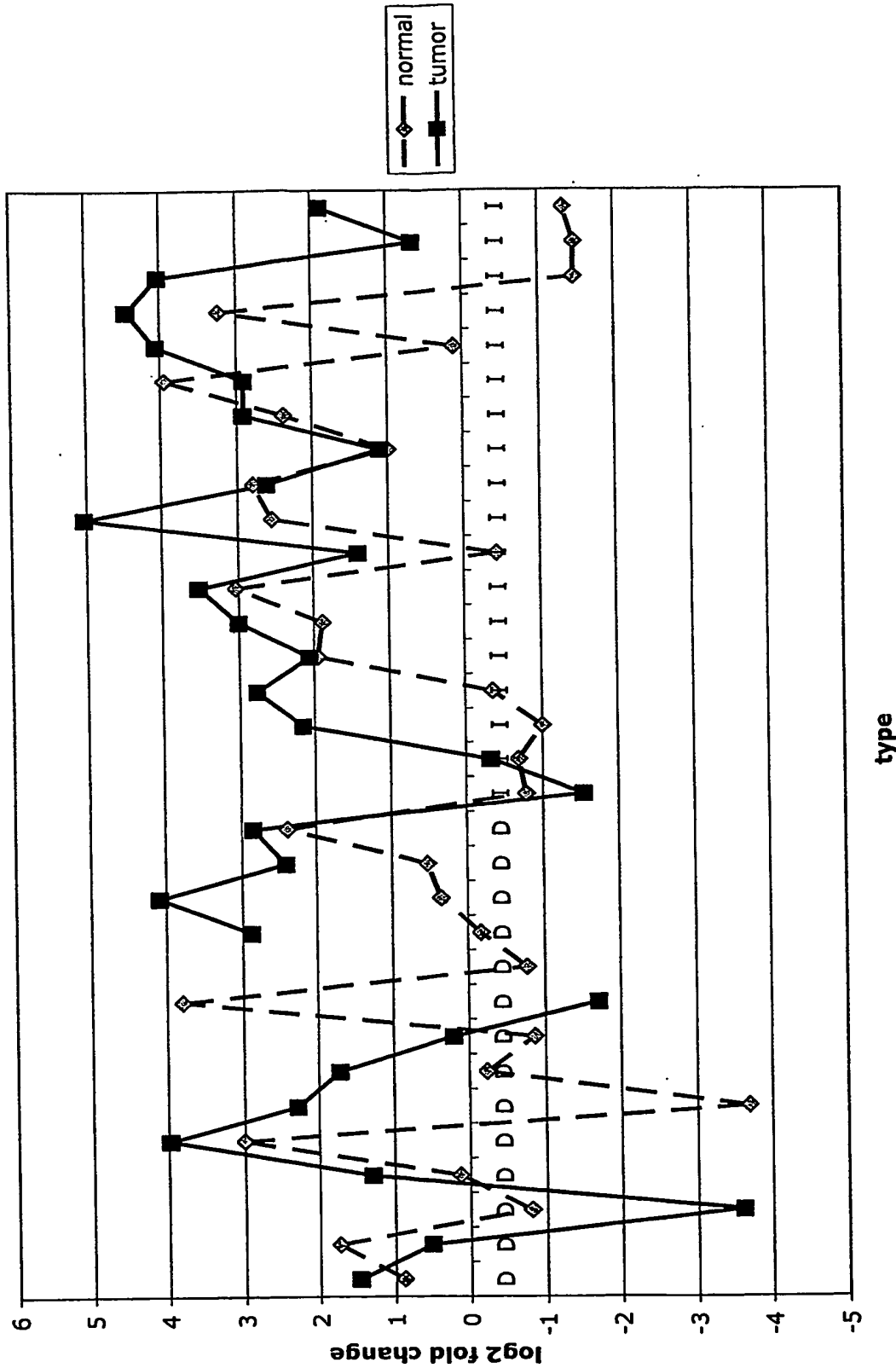




Fig. 11ab SERPINB5

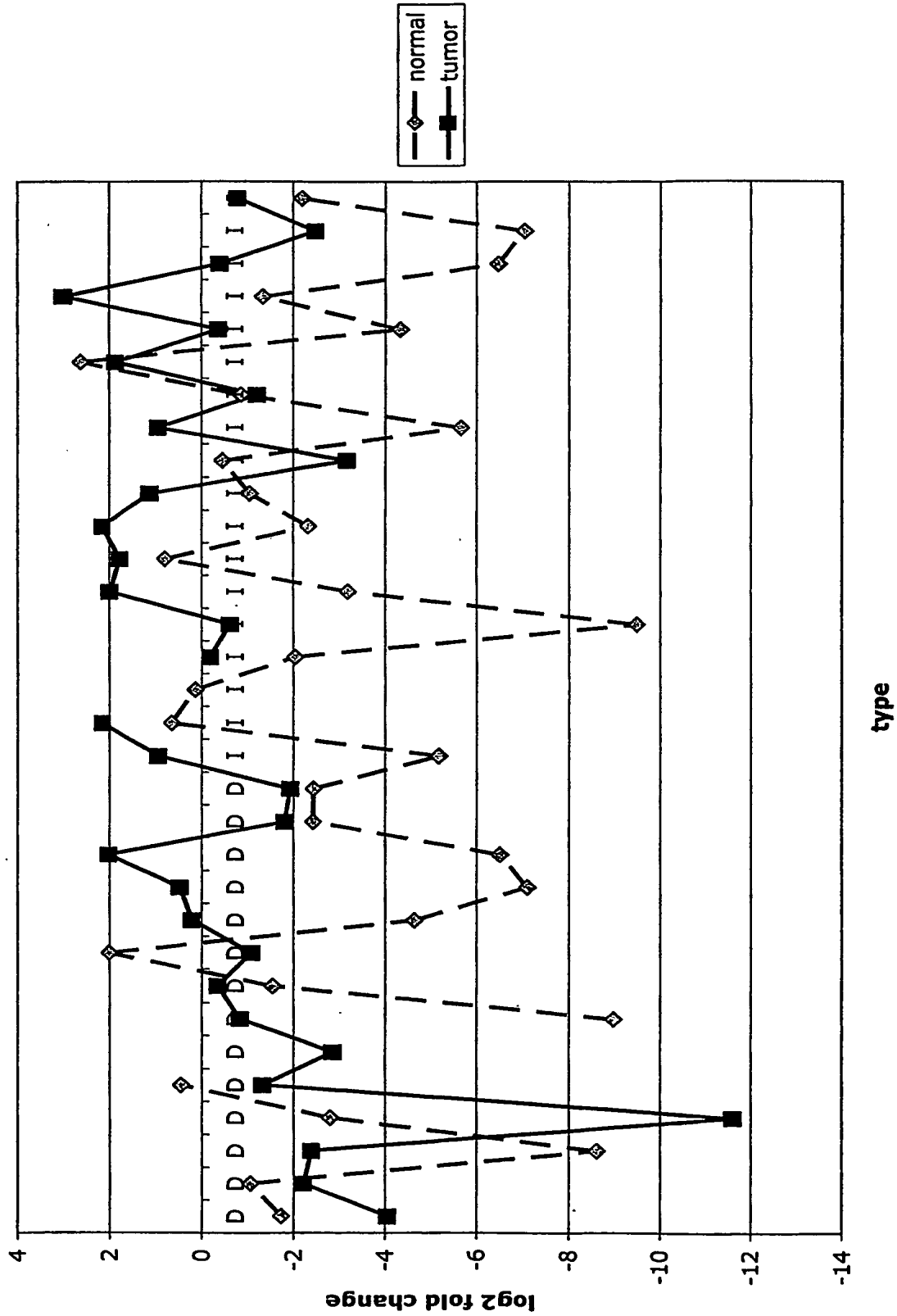


Fig. 11ac TGFB1

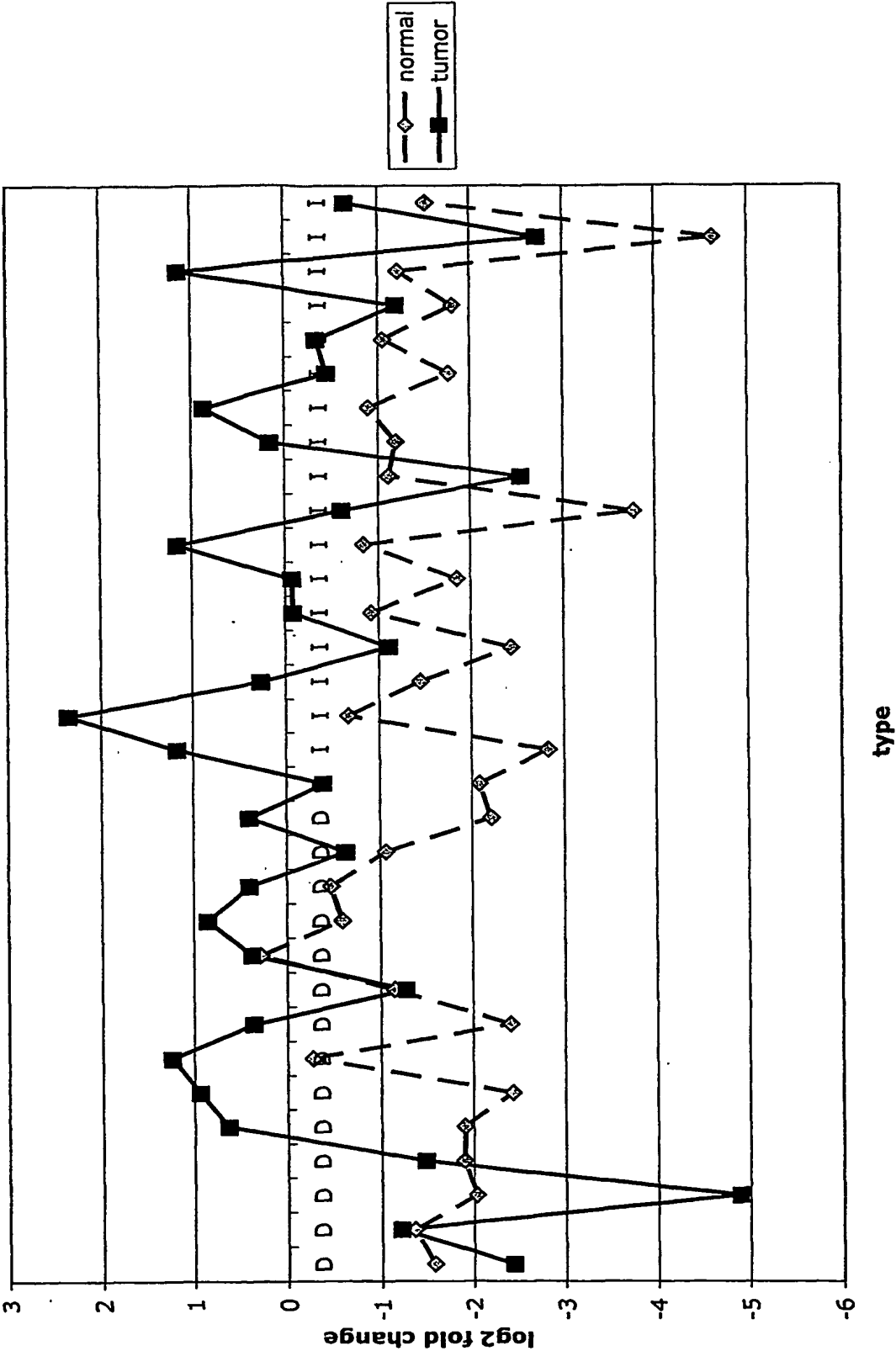
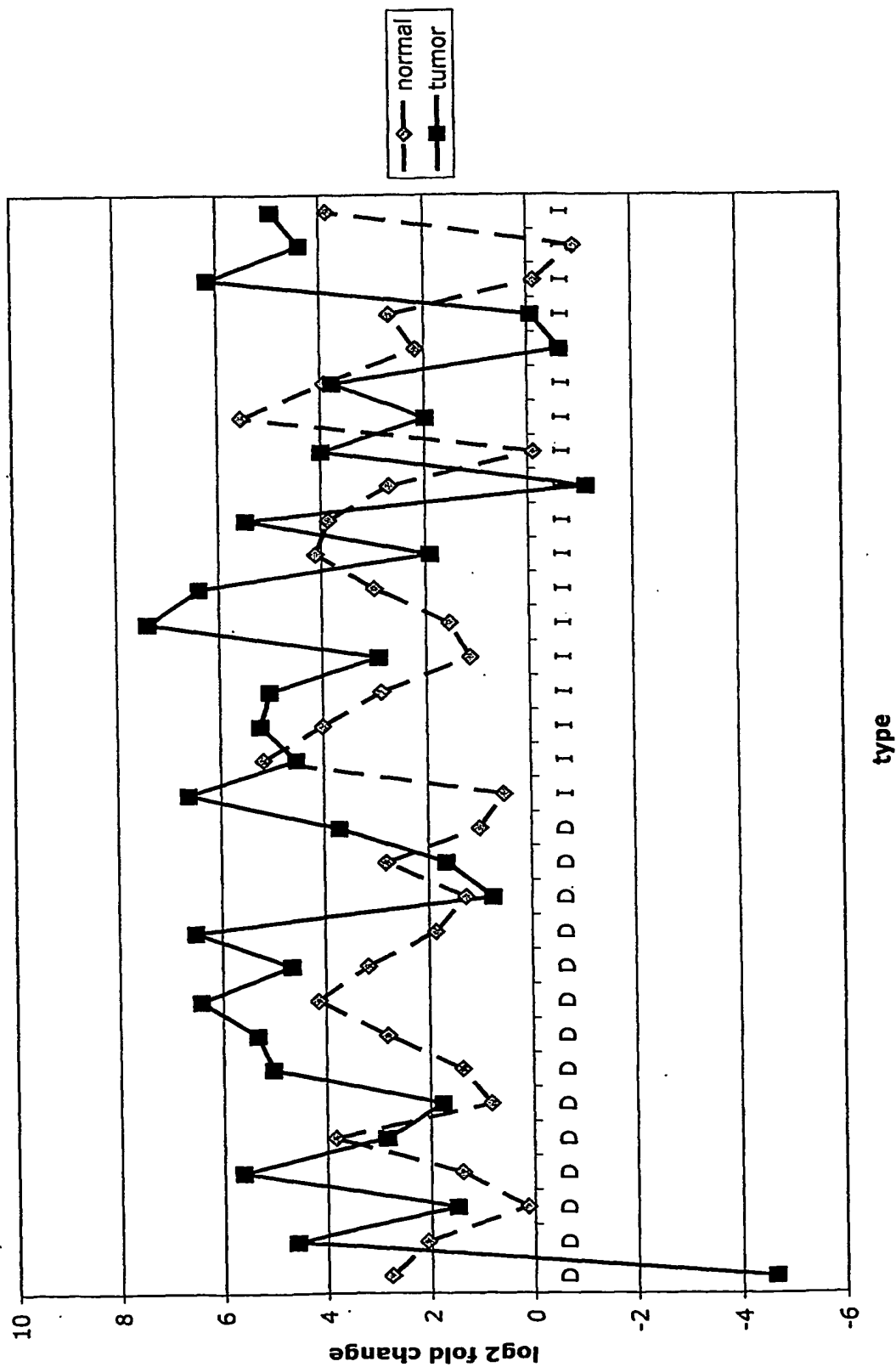
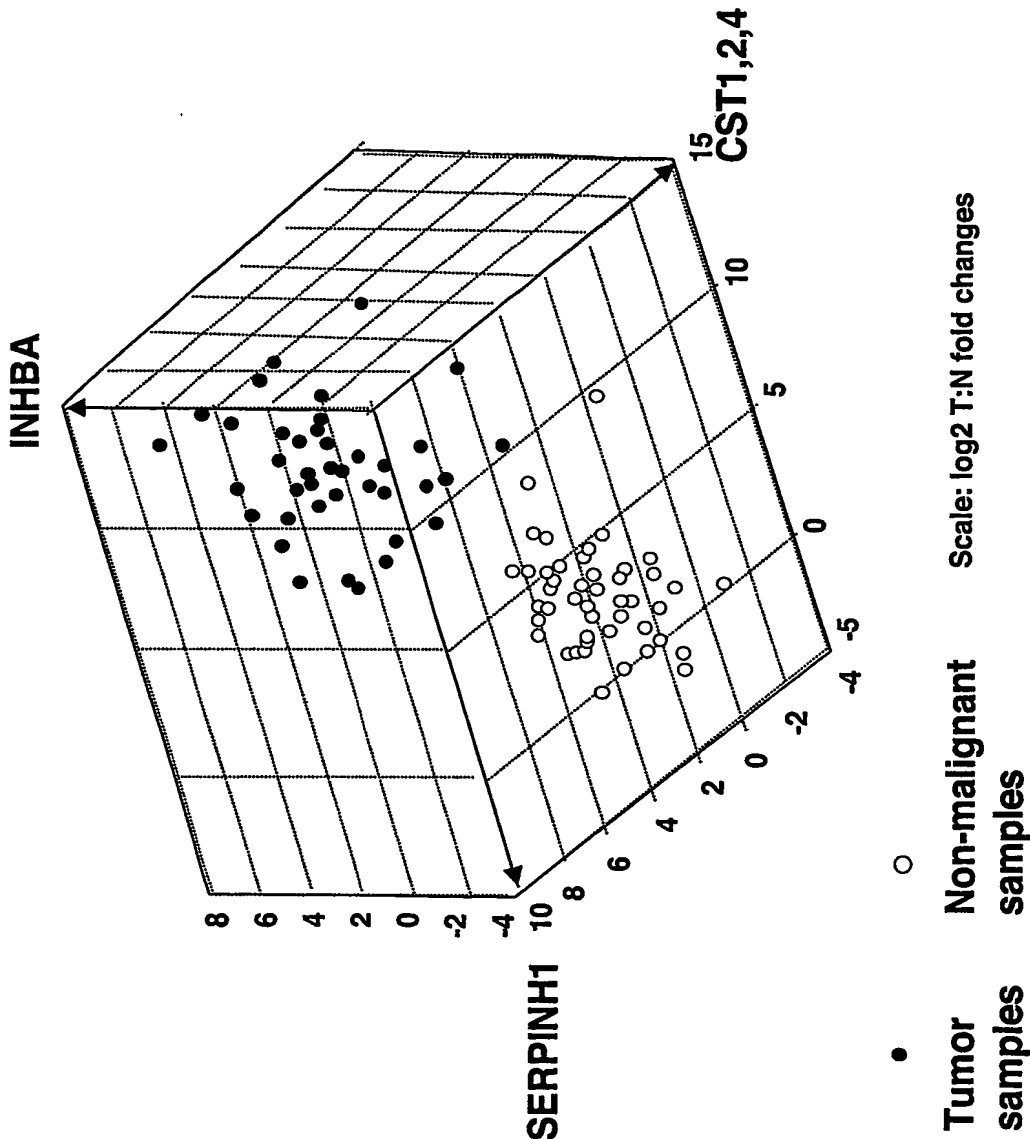


Fig. 11ad CEACAM5



**Fig. 12** The separation of gastric tumor samples from non-malignant samples using three markers



Number of markers in test	Total possible tests	Number of tests with sensitivity			Proportion of tests with sensitivity		
		>=90%	>=95%	>=99%	>=90%	>=95%	>=99%
1	29	2	1	0	6.9%	3.4%	0%
2	406	33	27	1	8.1%	6.7%	0.2%
3	3654	796	457	50	21.8%	12.5%	1.4%

**Fig. 13.** The effect of multiple markers on the ability to accurately discriminate between tumor tissue and non-malignant tissue.

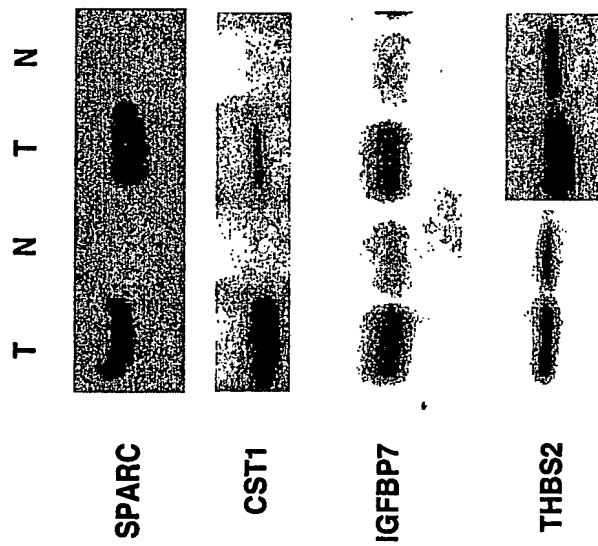


Fig. 14. Western analysis of markers in tumor and non-malignant tissue



**Fig. 15.** Western analysis of SPARC in gastric tumor material and serum.



**Fig. 16.** Immunodetection of cystatin SN in the supernatant of the gastric cancer cell line, AGS.